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That Was the Weak Force, That Was

FOR all that science is the great emergent culture of the twentieth century and that its ground rules are known to more educated men and women than those of any other cultural activity, it is still a strangely back-room affair as far as most members of the general public are concerned. There is more than residual belief that scientists should stick to science and that non-scientists can't be expected to understand a word of it. For this reason we have a lot to be grateful for in the offerings of BBC Television's *Horizon* as a long-running and generally successful attempt to portray a vast and comprehensible world of science in the terms of its human practitioners. We also have reason to appreciate the occasional televised *Controversy* debate as a sign that the scientist can have as important things to tell the public as do politicians, economists and administrators. But should we feel the same way about the occasional 2-hour-or-more programmes that BBC screens under Mr Nigel Calder's guidance? The double showing in Britain this week of *The Key to the Universe* has raised again nagging doubts that have surrounded Mr Calder's previous presentations: *The Violent Universe*, *The Mind of Man*, *The Restless Earth*, *The Life Game*, *The Weather Machine* and *The Human Conspiracy*.

It is Mr Calder's belief, put forward in *Nature* 245, 293 (1973), that close to the frontiers, communication of science to the layman is easier than in well-established areas because of the excitement, the starkness of the questions, the lack of jargon. Unfortunately, it is not equally evident that two hours is the right length of time to be spent on the subject, or that everything exciting within the subject should be covered. In the above-mentioned article Mr Calder himself described the problems of an overabundance of material, but he has often created the impression of not having pruned hard enough; the result has been a bewildering patchwork the underlying unity of which is not at all clear. In this most recent programme he managed to mention (in this order) neutrinos; the four forces; quantum mechanics; Feynmann diagrams; gravitons; gauge theory; Weinberg-Salam theory; renormalisation; 'heavy electrons' (muons); neutral currents; strangeness; the Ω -particle; quarks; colour; gluons; strings of gluons; anti-matter; exploding galaxies; radio background; big bang; lost symmetries; gravity 'frozen out'; latent symmetry . . . before the interval. We saw at least seventeen high-energy

or astronomical facilities. We were introduced colloquially to 'cosmic alchemy', 'pattern makers', the 'starbreaker', the 'fire-maker' force, our 'phantom friends', 'old man gravity', 'crazy ideas', 'spooky' black holes. We were treated to verbal infelicities like 'they are no luxuries, the electrical and mechanical aids at the Inter-Universities Institute for High Energies', and visual infelicities like the bizarre rope bridge from which Mr Calder declaimed, at times Messiah-like.

The initiated found a certain amount to admire in all this—new insights, nice turns of phrase, some clever visual work. The uninitiated, which must have been 99.9% of the audience, can only have been thoroughly confused by the profusion of jewels in the crown which were picked up in no apparent order, admired and put back, and with few concessions to those who blinked at the wrong moment.

Mr Calder and his team make one fundamental mistake: they underestimate the serious-mindedness of the audience. Viewers are not there by mistake or because they have been watching the previous programme; they are prepared to settle down for the required couple of hours to learn and many are even prepared to watch the programme twice. Such viewers don't need a breathless approach, they are prepared to devote time to fixing new ideas in their mind, if necessary by repetition, by schoolroom techniques and by relation to everyday experiences. They would also appreciate an historical account of why we are where we are; twenty minutes or half-an-hour of bringing the average viewer up to speed would indeed erode the number of exciting new concepts that could be crammed in, but it might ensure that the understanding of those concepts retained was more profound.

There is a serious danger in the presentation of science to the public that it should seem too esoteric and confusing. This widens the rift between scientists and non-scientists and does little to help persuade the young to turn to science. Mr Calder has consistently tried to avoid this by getting right amongst the discoveries as they come out, and in principle he is correct. But in practice the erudition he acquires in researching his programmes ends up overloading his audience, who may come to just the wrong conclusion that science indeed is somewhat too esoteric and rather too confusing for them. □



What role for fission?

Leslie Shepherd assesses the contribution nuclear fission can make to the energy requirements of the next 50 years

DURING the 1960s annual world energy demand was expanding at a rate of 4.5% a year. If that trend had continued, then 50 years from now the energy demand would be almost ten times as great as at present. With the emphasis that is now being set on the need for more careful and efficient use of energy, however, a lower rate of growth of perhaps 3.5% a year seems more probable for the future.

On this basis, 50 years from now we should be consuming primary energy at a rate of 1.5×10^{18} kilojoules a year and the cumulative consumption in those 50 years would be 3×10^{19} kilojoules. It is extremely unlikely that more than two-thirds of this could be met by fossil fuels because of the limitation on the total economic resources of petroleum oil and natural gas ($\sim 10^{19}$ kilojoules) and the feasible rate of extraction in the case of solid fuels. In fact, if the mining of coal and lignite were steadily increased by a factor of 5 over the next 50 years, the cumulative energy yield from these would be no more than 10^{19} kilojoules.

Nuclear fission, as the only practical alternative energy source to coal and oil on the scale which we are concerned with, therefore, may have to contribute at least one-third of the cumulative consumption by the year 2025 and thereafter provide the bulk of the future demand.

Uranium utilisation

Since uranium is the only natural source of fissile material, U-235, it is the basic raw material of nuclear power. One tonne of natural uranium contains sufficient U-235 to yield 5×10^{11} kilojoules of energy. The OECD's survey listing the reasonably assured and estimated additional resources of uranium from rich ores, published in December 1975, gives a current total of 3.5 million tonnes, the U-235 content of which, on its own, would yield only 1.75×10^{18} kilojoules. This is not a very impressive amount of energy—no more, in fact, than the present measured reserves of natural gas would yield.

Fortunately, the real reserves of fission energy, if they are properly exploited, are certain to be orders of magnitude greater than this figure

suggests. This is partly because exploration of uranium resources is still in its early stages and the actual amounts economically extractable are certain to be far greater than indicated in contemporary estimates. In addition, nuclear reactors not only consume the fissile U-235 charged into them, but also generate fresh fissile material by neutron absorption in the so-called fertile isotopes U-238 (which constitutes more than 99% of natural uranium) and Th-232 (the whole of natural thorium); this process of converting fertile to fissile material enables us to increase the fissionable content of nuclear fuel many fold.

The principal fissile isotopes resulting from these fertile captures are Pu-239 (from U-238) and U-233 (from Th-232). The amount of energy which we are able to derive from the originally mined natural uranium (and thorium) will be determined by the extent to which these secondary fissile isotopes can be generated and utilised starting from the primary U-235. This is a question of the 'neutron economies' of the various possible reactor systems and the fuel cycles adopted in them—the Uranium Cycle involving U-238/Pu-239, and the Thorium Cycle involving Th-232/U-233.

Not all of the neutrons absorbed in U-233, U-235 and Pu-239 cause fission; a proportion of the neutrons captured produce the higher isotopes U-234, U-236 and Pu-240, which in turn can absorb further neutrons yielding still higher isotopes. In fuels which have been in a reactor for some time the fissile components build up into a chain of isotopes (U-233, U-234, U-235, U-236 in the thorium fuel cycle, and Pu-239, Pu-240, Pu-241, Pu-242 in the uranium cycle). All of these isotopes have to be taken into account in evaluating neutron economy.

Neutron economy

Neutron economy refers to the balance of the processes which produce and absorb neutrons in a nuclear reactor. Each fission is the result of the absorption of a neutron in a fissile nucleus, and in the process fresh neutrons are emitted. One of these is required to produce a further fission, the balance going either into productive or non-productive processes. The productive process is neutron-absorption in the fertile isotopes, U-238 or Th-232, which yields new fissile material. Non-productive processes include:

- neutron-capture in the fixed materials making up the reactor core;
- absorption in fission products as they build up and in the control rods which are introduced to maintain the neutron balance as the fuel burns up;
- the non-fission neutron capture in the fissile species, which was mentioned above (the ratio of non-fission captures to fission captures is referred to as the α -value of the fissile species in question);
- neutron leakage from the reactor core.

It is, of course, the neutron absorption in fertile material, leading to the generation of fresh fissile isotopes, which is important, since it determines the ultimate amount of energy which may be derived from uranium and thorium resources. The ratio of fresh fissile atoms produced to those consumed in the chain of events described is known as the conversion ratio. If this is less than unity the amount of fissile material in the reactor steadily diminishes during operation and loss has to be made up periodically. However, if the ratio is greater than unity (it is then called the breeding ratio), there is a surplus of fissile materials available for other reactors and the system is said to breed.

Neutron economy varies according to the type of reactor and the manner in which it is operated; it also depends on the fuel cycle (uranium or thorium) adopted. The optimum situation is achievable in fast reactors, where breeding is possible largely because of the relatively low α -values in all the fissile materials when the neutron energy is high.

The uranium cycle is better than the thorium cycle in fast systems. This is because of the greater yield of neutrons in the fission of Pu-239 as compared with U-233, and because the most energetic neutrons can cause direct fission of the fertile U-238 (an effect which is far less pronounced in Th-232) and, by this means, boost significantly the number of available neutrons. In thermal reactors, the α -value is very high for Pu-239 (~ 0.6) and low in the case of U-233 (~ 0.1) with the result that much higher conversion ratios are possible with a thorium fuel cycle than with a uranium fuel cycle.

Thus from the standpoint of achieving the maximum energy output from natural uranium resources there is an incentive to develop fast reactors with the uranium cycle and to use the thorium fuel cycle in thermal reactors.

Thermal reactor characteristics

In a global context three categories of thermal reactor are of interest: light water reactors (LWR), heavy water reactors (HWR) and the helium-cooled

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high temperature reactor (HTR).

As far as neutron loss in the moderator is concerned, light water is worst, carbon is intermediate, while heavy water is essentially non-absorbing. The same remarks apply to light and heavy water as coolants; no neutrons would be absorbed in a helium coolant. LWR and HWR cores both incorporate a large amount of neutron absorbing metal in fuel element cladding and pressure tubes, so that these systems are appreciably worse in this respect than the HTR which includes no metal structure. Taking these factors into account both the HTR and the HWR have an advantage over the LWR.

The proportion of neutrons lost by absorption in fission products (more or less independent of reactor type) depends upon the extent to which these are allowed to build up relative to the core inventory of fissile material, that is to say the degree of fuel burn-up before it is discharged from the reactor for reprocessing. Reprocessing separates out the fission products from the unburned fissile material which can then be recycled in the reactor. Reprocessing is an expensive matter and for that reason among others it is desirable to keep a high inventory of fissile material in the reactor core (if a good neutron economy is desired) to avoid too frequent recycling.

The yardstick in the evaluation of future thermal reactors is the current uranium fuel cycle LWRs. These operate at present with no recycle. The conversion ratio in the single fuel cycle is about 0.60, and more than half of the Pu-239 produced is actually consumed in this cycle. An LWR of 1000 MW gross capacity, operated in this way for 30 years at 80% load factor, would require 6,000 tonnes of natural uranium. At this rate about 25 million tonnes of uranium would be required to yield the 10^{19} kilojoules indicated above as a cumulative energy target over the next 50 years. This amount could be reduced by about 40% if fuel were recycled to use up all residual U-235 and plutonium.

Both the HWR and the HTR would achieve a better performance than the LWR with the uranium fuel cycle for the reasons already given. In the case of the HTR, the superior thermodynamic efficiency of the power conversion cycle*, arising from the higher temperatures achieved, would also mean that the useful energy ultimately derived would be substantially higher than with the water cooled reactors.

The biggest gain, however, would come from the use of the thorium fuel cycle in thermal reactors because of

the greater yield of neutrons from U-233 compared with Pu-239. This would carry a potential advantage of about 0.4 in conversion ratio compared with the uranium cycle LWR and even introduce the possibility of operating thermal reactors as breeders. However, this would require very large inventories of fissile material in these reactors so that the initial large requirement of mined uranium would offset the advantage of the breeding for a very long period, certainly longer than the 50 years considered here.

Furthermore, U-235 being the only naturally occurring fissile material, all nuclear fuel cycles must begin with this isotope. This means that there is an initial phase in which the neutron economy is determined partially by the characteristics of U-235, with a consequently lower conversion ratio during that phase.

High conversion thorium fuel cycle thermal reactors would require some 5-7 million tonnes of uranium to be mined over the next 50 years in order to achieve an energy output of 10^{19} kilojoules. This is in excess of the presently estimated resources of uranium from high grade ores. Without doubt, the present figures greatly underestimate the actual available resources, particularly if one takes into account the acceptability of using much more costly uranium in high conversion or breeding systems.

At the end of that time, however, at the 3.5%-a-year rate of energy growth the annual nuclear energy demand would be $\sim 10^{18}$ kilojoules and would be increasing. It is difficult to envisage meeting this requirement without having built up an adequate breeder reactor capacity. This provides

the incentive to develop a technically and economically viable fast reactor and to have such a system established on a substantial scale 50 years from now.

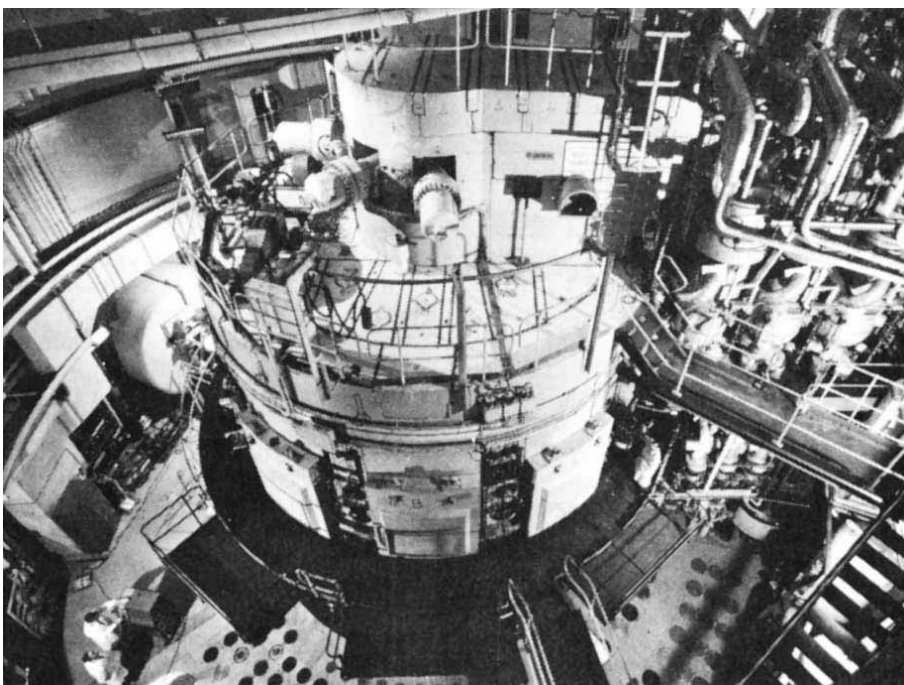
Fast reactor exploitation

A fast reactor using plutonium will have an initial induction phase in going from U-235 to Pu-239, as would be the case in building up a U-233 inventory in a thermal reactor. Currently, the accepted strategy is to use thermal reactors (predominantly LWRs) to produce the necessary plutonium inventories for fast breeders. Unfortunately, this is a particularly inefficient process, a non-recycle LWR yielding only about a quarter of a kilogramme of fissile plutonium per MW-year.

Taking four tonnes as being a minimum plutonium inventory for a sodium-cooled fast breeder of 1000 MW capacity (2.5 tonnes in the core and 1.5 tonnes in recycle), its production would require an LWR of corresponding power output to operate for 20 years at 80% load factor, consuming more than 3,000 tonnes of uranium in the process.

Subsequently, the fast reactor would yield excess plutonium which would be available to build up further inventories. There is a possibility, however, that the doubling time—that is to say the time for the fast reactor to generate sufficient excess plutonium to provide a further reactor inventory—may be very long, which would make for an excessively slow build-up in the ratio of fast reactors to LWRs.

In such circumstances there would be, certainly over the next 50 years, an unacceptably high preponderance of



A view of the Dragon

*An electricity generating HWR would have a thermal efficiency of 30% compared with 40% for a steam cycle HTR and 45-50% for a binary cycle HTR.

LWRs over FBRs, so that one would be far removed from an overall breeding situation and the total uranium demand could be significantly higher than for a programme based only upon high-conversion thorium fuel cycle reactors.

This approach is unnecessary, however, it being much more logical to start fast reactors with U-235 and produce the eventual Pu-239 inventory *in situ*. In this way all of the plutonium would be produced and consumed in its most favourable environment. The time to produce a full plutonium inventory and, therefore, a true breeding situation would be much shorter and the uranium input very much reduced, possibly by a factor of four, compared with the LWR approach.

A further advantage of this course is that it would make the fast reactor independent of any thermal system, and it would relieve the latter of the requirement to work on the uranium fuel cycle in order to provide plutonium for fast systems.

While thermal reactors are not appropriate sources of plutonium for

fast reactors, it is worth stressing that the converse is not true, insofar as U-233 could be generated efficiently in thorium introduced into the blanket of a fast breeder reactor. In this way a fast reactor with a good breeding ratio could support high conversion thermal reactors with a combined energy output several times greater than its own.

This is an important possibility leading to a symbiosis of a small number of fast breeders with a larger number of high conversion helium-cooled high temperature reactors, which would be preferred to the fast reactor on all counts other than neutron economy.

Conclusion

In the foregoing a world viewpoint has been taken deliberately, because no individual nation can hope to proceed in an isolated manner in resolving problems of such significance as those presented by the energy situation.

Unless some viable major alternative energy source (such as nuclear fusion) comes along, there will be a minimum cumulative requirement of 10^{19} kilo-

joules of thermal energy from nuclear fission over the next 50 years, and at the end of that time the annual energy contribution will need to be 10^{18} kilojoules a year. The requirement is far beyond the capabilities of light water reactors working on their present uranium fuel cycles, but it would not be beyond the capacity of thorium fuel cycle thermal reactors, notably the high temperature reactor, provided that they were being followed up with a well established programme of fast reactors.

However, the strategy of using thermal reactors (predominantly LWRs) to produce the plutonium inventories needed for breeding does not appear to be viable. The rapid exploitation of the breeding capabilities of fast reactors requires that they should generate their own plutonium from the initially available U-235. On this basis the evolution of fast reactor exploitation would proceed independently of the programme of thermal reactors. Eventually, the two would come together in a symbiosis, in which an overall breeding situation would be maintained with a minimum of fast reactors. □

FRANCE

Debating nuclear power

Alexander Dorozynski reports from France on the nuclear power debate there

THIS year may be decisive for the ambitious French programme of rapid development of nuclear energy. Well before the debate triggered by the "all nuclear" decision of the Pierre Messmer government in March 1974 physicists and ecologists started voicing worries about the programme's safety and reliability, and the doubters appear still to be growing in numbers and in political weight.

The controversy has spread beyond the potential dangers of a technology that has not been entirely mastered. Those arguments find a focus in the huge nuclear waste treatment plant in La Hague, at the tip of the Cotentin directly south of Bournemouth, and in the Superphénix fast breeder reactor, to be located just 44 km from Lyon. The debate now extends to many of the economic and political arguments advanced by the powerful Electricité de France (EDF), the state monopoly employing some 100,000 persons and one of France's largest and most powerful enterprises.

The Ministry of Industry and Research has been only marginally

effective in dealing with the 'dissidents', and in the face of mounting public awareness, a public relation campaign is being mounted by EDF. Embarrassingly, some of the approaches to be taken have been revealed by an ecological weekly, *La Gueule Ouverte*, which has published a confidential EDF document giving recommendations about how to deal with different categories of opponents, such as the public at large, members of the parliament, physicians, teachers, journalists, and so on.

Now another publication, *Science et Vie*, France's largest science magazine, is publishing the results of a debate it has organised about the economic soundness of the programme. The debate put face to face economists from the EDF and members of the Institut Economique et Juridique de l'Energie (IEJE), a small group formed at the University of Social Sciences of Grenoble. A guest participant was Professor Irvin C. Bupp, Jr, a Harvard University economist who has taken part in a MIT study of the evolution of the price of water reactors in the US—PWRs are being installed in France under a patent agreement with Westinghouse.

The initial nuclear programme has already been toned down under Valéry Giscard d'Estaing's presidency:

annual plant construction will be decreased from a 6,000 MW capacity in 1976 and 1977 to 5,000 MW in 1978, and the goal of producing by 1985 nuclear energy equivalent to 60 million tons of oil has been adjusted downward to 55 million. But until now, few had challenged the figures authoritatively produced by EDF concerning the price of the nuclear kilowatt, and the cost of nuclear plants.

"The only economists who have ventured to challenge official figures are those of the IEJE," points out one participant, Jean Marie Chevalier, professor of economics at the University of Paris-Dauphine. "In fact, the nuclear programme has been imposed upon the country by EDF and the Commissariat à l'Energie Atomique (CEA). Never has there been the slightest counter-expertise. The government let it go without compromising itself. Thus, if need be, it will be able to reject upon EDF the responsibility of the aberrant situation we might end up with." The figures are now likely to be subjected to close scrutiny.

For instance, EDF economists have tagged the nuclear KWh with a cost of 7.5 centimes, while that of the 'classical', oil-fired KWh varies between 11.5 and 12.5 centimes. IEJE economists ask if this price is realistic. Just across the border, in West Germany, the nuclear KWh is priced at 6 pfennig (12 centimes), and Patrice Romain of the IEJE maintains

that if engineering costs, insurance, cost of processing waste and of dismantling reactors are included, the EDF price goes up steeply. Then there is the cost of uranium, which has gone up from \$5 a pound in 1973 to \$40 a pound. Nuclear fuel cost, once estimated at 20% of total costs, has now reached 30%, and is still growing. If waste treatment is included, notes Jean-Marie Chevalier, it may reach 60%.

Similarly, the cost of constructing nuclear power plants is challenged. Is it closer to the FF2,150 per installed KWh, as quoted by EDF, or to FF6,700, the price France would like to charge for similar installations in Iran? Does this difference mean that a cost in constant francs must be upped by 50–100% for a foreign country, that more safety factors are included for export, or that EDF discounts some hidden costs that appear elsewhere in its budget? In the United States, Bupp notes, this cost was \$130 per installed KWh in 1965, \$300 in 1972, and is estimated today at some \$700 (about FF3,500).

Another major argument frequently advanced for the intensive development of nuclear power is also being challenged: that of energy independence. According to a report to the National Assembly, French requirements in uranium from now to 1985 are estimated at 85,000 tonnes, and by 1990 at between 115,000 and 125,000 tonnes. "If France had to count on its own resources, these would be exhausted between these two dates," states the report. France may have interests in mining operations in Niger, Gabon and Canada, but it is wondered if there is a guarantee that these interests will be preserved.

There are questions, too, about France's real independence with regards to enrichment. France's uranium enrichment plant at Pierrelatte, designed for military purposes, has insufficient production capacities. The Tricastin plant nearby should only be operative in 1980—if all goes well.

None of the 'dissidents', whether physicists or economists, argue against nuclear power as such. Their major objection is that the EDF plans for producing energy from the atom and using electricity as a vector have closed the door to alternatives. In 1977, only FF30 million will be devoted to research in new energy sources, as against FF2,474 million budgeted for CEA projects.

"We are making a mistake by not playing the card of natural gas," adds Chevalier. "Gas is found everywhere—in North Africa, the Middle East, the Soviet Union, Holland, under the North Sea. It is energy which, unlike oil, is



Reprocessing plant at La Hague

not dominated by multinational firms. . . Contracts between state and state could be made to preserve equitably the interests of both parties. And gas is a decentralising energy, unlike nuclear power, which leads to the development of maxi-sites and of national distribution networks. With gas turbines, we can produce locally and on a small scale electricity and heat, thereby reducing social costs."

Chevalier believes that no other form of energy can be made available as rapidly as gas, and that within four years, a flow could be created that would represent a saving of FF25–35,000 million. Meanwhile France, which imports three-quarters of the energy she consumes, could explore alternatives before the nuclear 'solution' becomes irreversible. □

● Azim Kidwai writes from Karachi:

Although nuclear cooperation between Canada and Pakistan has ended (see *Nature*, January 6, page 9, and also January 13, page 95), the deal with France for the supply of a nuclear reprocessing plant still stands. The plant was one of the main bones of contention in the dispute with Canada. Considerable pressure on Pakistan to cancel the French deal also came from the USA. Pakistan defended the right of two sovereign states to enter into such agreements, but to allay the fears of those who thought that the plant would be used for other than peaceful purposes, it showed its willingness to negotiate further safeguards even though the agreement had been cleared by the International Atomic Energy Agency.

The ending of Canadian assistance will have its effects. The only nuclear power plant running in Pakistan, the 137 MW Karachi nuclear power plant (KANUPP), was supplied by Canada. Its fuel, essential spare parts and the technical assistance for its maintenance were from Canada. A policy of self-

reliance may now be the order of the day, and the Chinese experience when Russian technical assistance was withdrawn in the early 1960s is being cited.

The supply of fuel to KANUPP may pose no problems, as fuel fabrication is likely to be arranged from sources other than Canada. Eventually fuel is to be fabricated in Pakistan itself, and had Canada not, two years ago, stopped the shipment of equipment for fuel fabrication from Canada to Pakistan, the fuel for KANUPP would now be locally available. Considerable uranium bearing sandstones have been discovered in the northern areas of Pakistan in the Dera Ghazi Khan district and a survey of uranium bearing ores has been completed with the assistance of the International Atomic Energy Agency. Plans are in hand for their exploitation.

KANUPP feeds into the Karachi grid only 85 MW on average, and although its closure for long periods could be a source of irritation a 125 MW steam-powered generating unit is under installation, to be commissioned by July next. Another five gas-turbines each of 25 MW have also been urgently ordered for Karachi as a further reserve to be commissioned by July 1978.

The Pakistan government called the Canadian action arbitrary, adding that it violated three bilateral agreements on cooperation with Pakistan in peaceful uses of atomic energy. She found unreasonable the new conditions for which Canada was asking. One was that Canadian safeguards "will cover Pakistan's entire nuclear programme, not merely the nuclear facilities provided by Canada". Pakistan also found unacceptable the demand that even if "Canada should terminate all nuclear cooperation with Pakistan under the bilateral agreements, Pakistan shall nonetheless remain bound by its commitments under those agreements for the rest of the operating life of KANUPP". □

USA

Carter's energy problem

As America freezes energy matters grow more important. Colin Norman reports from Washington on the continuing search for an energy policy

It didn't take long for President Jimmy Carter to be brought face to face with the energy crisis. Within a week of taking office, he was forced to ask Congress for emergency powers to deal with a critical shortage of natural gas

in parts of the eastern United States—a shortage brought about by an extraordinarily cold winter and several years' lack of a coherent energy policy. Though at this stage Carter can do little more than spread the shortage around, urge people to be less profligate in their use of energy and pray for warmer weather, the new Administration is promising to set things right in the long term with a comprehensive energy policy, a strong conservation plan and an overhaul of the federal

energy bureaucracy.

Clearly, energy matters are going to keep the Administration busy, but at least it will have no shortage of outside advice. In that regard, a mammoth study now being put together by the National Academy of Sciences is likely to prove particularly influential. Due to be completed by June 30, the study is one of the most ambitious projects the Academy has yet undertaken. It involves some 250 people, sitting on a variety of panels and committees, who are now channelling their advice to a steering committee co-chaired by the ubiquitous Harvey Brooks, professor

Changing of the guard

● As the new Carter Administration settled into its first full week in office, selection of the second-level Presidential appointees began in earnest, but by the end of the week, none of the key science posts had been filled. The most important job, head of the Office of Science and Technology Policy (OSTP) and Science Adviser to the President, was still open following the departure of H. Guyford Stever on January 20. About the only sign of movement is that Carter has asked the Defense Secretary, Harold Brown, to draft a list of potential candidates for the OSTP post, a curious assignment since OSTP has little influence over defence matters, and most other Presidential appointments are being handled by a team in the White House.

As for the major science agencies, Donald Fredrickson, the popular director of the National Institutes of Health (NIH), had not heard by the end of last week whether he will be kept in his job. At a press conference, however, Joseph Califano, the new Secretary of Health Education and Welfare, promised to end the politicisation of NIH, but he said he hadn't made up his mind about Fredrickson's future. Similarly, Robert Seamans Jr, has departed as head of the Energy Research and Development Administration, but no successor has been named. It is widely reported, however that Douglas Costle, a former staff member of the Congressional Budget Office has been chosen as head of the Environmental Protection Agency, to replace the already-departed Russell Train.

About the only major scientific appointment so far is that of Patsy Mink, a former Congresswoman from Hawaii, to be Assistant Secretary of State in charge of science and technology affairs. The post was once held by Dixy Lee Ray, the former

head of the Atomic Energy Commission who is now Governor of Washington State, but she quit with a blast at Henry Kissinger because of his indifference toward her office. The selection of Mink, a lawyer, for a post which many people consider should be held by a scientist, or at least by somebody well versed in scientific matters, will not do much to enhance the status of the office.

● Meanwhile, at the other end of Pennsylvania Avenue, Congress is finally getting itself in gear for the new session. The plan to reorganise the committees of the Senate, which was drafted last year by a special select committee, has now been picked over and amended by the Senate Rules Committee and it is expected to be debated by the full Senate this week. Although several more changes are anticipated, the chief committee alignments for science and technology affairs are likely to remain virtually as proposed by the select committee.

The Joint Committee on Atomic Energy, which has already been cut up by the House, will be completely done away with, and all jurisdiction for energy policy and energy research and development will be placed in an expanded Interior committee, under the chairmanship of Senator Henry Jackson. Responsibility for biomedical research will be consolidated in a new Committee on Human Resources, based on the old Labor and Public Welfare Committee, which means that Senator Edward Kennedy's subcommittee will retain its jurisdiction. The rest of the responsibility for science and technology, including overall science policy affairs, will be placed in an expanded Commerce Committee. Senator Adlai Stevenson, who chaired the select committee, is expected to end up with chairmanship of the key Commerce subcommittee dealing with

science and technology.

The only major change from the select committee's proposals is that jurisdiction over the National Science Foundation (NSF) will go to the Human Resources Committee. On the face of it, a decision to put NSF affairs into a committee which deals with health and welfare rather than into the Commerce Committee, which deals with science, may seem a bit illogical. The reason is that Kennedy, whose NSF subcommittee now handles NSF matters, is a member of the Human Resources Committee but not of the Commerce Committee, and he wants to hang on to his NSF jurisdiction. That's the way Congress works.

On the House side, the Science and Technology Committee, which has picked up responsibility for nuclear research and development from the Joint Committee on Atomic Energy, has been reorganised. Chairmanship of the subcommittee on Science, Research and Development, which deals with NSF, has been assigned to Representative Ray Thornton, a Democrat for Arkansas. Energy matters will be handled by two subcommittees, one of which will deal with fossil energy and nuclear fission and fusion, while the other will deal with solar and geothermal energy, energy conservation and basic energy research. Rep. Walter Flowers, a long-serving member from Alabama, claimed the chairmanship of the fossil and nuclear subcommittee, while Representative Mike McCormack, who was widely expected to get authority over nuclear matters, was left with the solar and geothermal subcommittee. Flowers' views on energy policy are little known, but his new post will give him plenty of visibility, a useful asset if, as expected, he decides to run for the Senate in 1978.

Colin Norman

of technology and public policy at Harvard, and Edward Ginzton, chairman of Varian Associates.

A tantalising preview of some of the conclusions likely to emerge from the study surfaced last week when the steering committee issued an interim report, essentially suggesting that energy use in the United States will grow at a slower pace than most other studies have anticipated. The committee suggests, moreover, that the country can adjust to a slower rate of energy growth without sacrificing economic or social goals.

If the Academy does indeed come up with such a conclusion, it would add considerable weight to the view put forward a couple of years ago by the Ford Foundation's Energy Policy Project, namely that energy growth can be gradually reduced over a decade or so to a very low rate, without either causing economic stagnation or radically altering people's lifestyles. The practical consequence of such a development—if true—would be that there may be much more flexibility in long term energy supplies than is generally realised; Mr Carter could, for example, carry out his campaign pledge to slow down the present breakneck pace of the breeder reactor programme, without running a large risk of causing massive economic disruptions toward the end of the century.

Further evidence of the growing acceptance of the view that energy growth is likely to slow markedly is

also to be found in a meticulous study of the possible consequences of a nuclear moratorium, produced recently by the Institute for Energy Analysis (IEA), headed by Alvin Weinberg. The study predicts that total energy use in the year 2010 will lie between a low of about 118 quads and a high of about 159 quads, compared with 71 quads in 1975. It notes that "even our 'high' estimate . . . is much lower than most previously published estimates. If our estimates are valid, they could imply considerable rethinking of those elements of energy policy and energy R&D policy that are premised on higher overall projections."

Because of those lowered energy growth rates, the study estimates that the economic and environmental impacts of a nuclear moratorium (which would prohibit the construction of new reactors after 1980 but allow continued operation of reactors on line by 1985), though severe, would be smaller than is generally believed. It is assumed that most of the energy shortfall would be taken up by a switch to coal in the short term, with growing input from alternative sources such as solar energy, which would tend to increase the cost of energy by a total of between \$31,400 and \$42,000 million by the year 2010, the study estimates. That would amount to less than 1% of the gross national product each year. As for employment, the study predicts that a moratorium would result in the loss of about 50,000 jobs in the nuclear and

related industries, but "the displacement caused by the moratorium would be temporary".

The environmental implications of a switch from nuclear to coal could be relatively severe. If the moratorium is coupled with limitations on oil imports, which would be likely, between 100 and 300 million more tons of coal would have to be mined each year in the period between 2000 and 2010 than would be the case without a moratorium. That would probably despoil larger areas of land for strip mines, increase atmospheric levels of carbon dioxide, and possibly increase pollution from sulphur dioxide, oxides of nitrogen, hydrocarbons, particulates and carbon monoxide. Increased use of pollution control technologies would, however, take care of some of the increases.

Though such impacts are relatively large, they certainly do not add up to the level of economic and social disruption predicted by the nuclear industry last year during the referendum in seven states on nuclear power.

The expectation that energy growth in the United States will slow down over the long term without precipitating economic stagnation, which drew sharp criticism when the Ford Foundation published its study, is therefore growing in acceptance. It means that President Carter may have more flexibility when he chooses his long range energy plans, but it won't help much over the next few years. Only some warmer weather would do that. □

AUSTRALIA

SCORE scores

Peter Pockley reports from Sydney on Australia's data collecting project for research expenditure

INACTIVITY on questions of priority and level of financial support for science in Australia has regularly been justified on grounds of a lack of reliable data on expenditure and manpower. In the early 1970s, the then Department of Education and Science started the first national data collecting operation and graced it with the acronym of Project SCORE (Survey and Comparison of Research Expenditures).

The project, however, was for some years plagued by problems of definition, management and staffing. But it did manage to grind out in 1973 a detailed set of figures for the financial year 1968–69 (the Australian financial year runs from July 1 to June 30, with the exception of the universities whose accounts run from January 1 to December 31—just one of the problems faced

by the number crunchers). These figures came out just as inflation began to canter up towards the gallop of 1975 and 1976, and this, coupled with the long delay between year of study and year of publication, greatly diminished their value.

The Department of Science has now begun publishing the second set of Project SCORE data. These refer to the financial year 1973–74; being more recent, the project scores more useful points for the student and politician of science alike. However, the criteria used in collecting and processing the data are not entirely consistent with the 1968–69 figures because the project changed to following OECD prescriptions very closely. This makes international comparisons valid, but has negated any reliable and precise statements on national trends in expenditure and manpower which are so urgently needed for drumming complacency out of politicians. This will have to await the next publication planned for the financial year of 1975–76 (the Depart-

ment now hopes to collect data at two-yearly intervals).

Project SCORE reveals that in 1973–74 the Gross Expenditure on Research and Development (GERD) amounted to 651 million Australian dollars (£1=\$1.58). This represents 1.3% of the Gross Domestic Product (GDP) of \$50,557 million for the year under study. The manpower involved in GERD was equivalent to 53,300 man-years, representing 0.9% of the Australian work-force of 5,867,700; 48% of the manpower effort was attributed to professional persons. There are, however, no figures for the supply and demand of professionals, nor are these available from other sources to confirm or deny the now popular belief in Australia of an oversupply of scientists and technologists at a time when overall unemployment is nudging 5% of the workforce.

Using OECD's definitions, 91% of GERD was spent in the natural sciences, and 9% in the social sciences and humanities, assuming all business enterprise expenditure to be in the former. Basic research took 28% (\$184 million) of GERD, applied research

37% (\$238 million) and experimental development 35% (\$229 million).

Socio-economic objectives groups accounted for intramural expenditure as follows, assuming all expenditure in the business enterprise sector to be in the economic development group: economic development 59%, advancement of knowledge 26%, national security 9%, and community welfare 6%. These and other broad figures quoted are broken down into fine detail in Project SCORE's publications.

As a percentage of GDP, Australia's GERD (natural sciences only) was in the middle rank of OECD's 18 member countries for the calendar year 1973. Australia's figure, adjusted to that year, was 1.2%, compared with the USA

2.3%, UK 2.1%, Germany 2.0%, Netherlands 1.8%, Japan and France 1.7%, Sweden 1.6%, Belgium 1.3%, Canada and Norway 1.1%, Austria 1.0%, and New Zealand 0.9%.

Changes in Australian R&D expenditure in the five years from 1968-69 to 1973-74 are difficult to quantify because of the changed criteria. Project SCORE's published figures for 1968-69 put total GERD at \$334 million, approximately half the 1973-74 figure of \$651 million without taking inflation into account. If compared at constant prices, GERD has increased about 35%, but when measured as a percentage of GDP, the GERD figures are closely comparable.

The relative financial support of

Australian R&D has remained fairly static over the five-year period between comprehensive data collections and analyses. The figures thus support the general contention from more superficial indicators that neither the Liberal nor Labor governments of recent times gave significantly more or less attention to science than to other areas of government finance. Using the superficial indicators for the period since 1973-74, it is at least certain that the relative standstill position has been maintained, and quite likely that there has been a significant slippage. For example, it is believed that the number of staff engaged on industrial R&D has dropped by as much as 40% over the two years from 1973-74. □

IN BRIEF

Gloomy energy report

In a sombre analysis released last week of projected global energy supply and demand to 1985, an OECD publication, *World Energy Outlook*, warns of a likely shortfall in oil supply that will mean either higher prices or shortages or both. The report, calling for a "transformation" and "revitalisation" of energy policies in industrial countries, urges them to increase indigenous energy production and improve conservation through a combination of financial measures, including investment incentives and use of the price mechanism, and regulations, embracing direct and indirect conservation measures and relaxed environmental requirements.

Brazilian deal developments

Reports in Brazilian papers suggest that Brazil is determined to resist pressure from the United States to change the terms of its controversial nuclear deal with West Germany. There are also signs that Argentina, which has its own nuclear ambitions, is proffering support for the stand. The reports follow others in the Brazilian press indicating a possibility that the US might consider supplying Brazil with nuclear fuel if it gave up that part of the German deal which includes both enrichment and reprocessing facilities.

The US Vice-President, Walter Mondale, apparently urged West Germany to restrain sales of nuclear technology when he visited Bonn last week as part of his diplomatic tour of key western capitals, and the possibility has emerged of including tighter curbs on trade in sensitive technology. Nothing specific was disclosed regarding the Brazilian deal, however.

Brazil has not signed the nuclear Non-Proliferation Treaty, and although the deal is permissible under the treaty,

it is hardly in keeping with President Carter's intentions in the field of international trade in nuclear technology.

Safety bodies established

The UK Health and Safety Commission has announced the establishment of two more bodies which are to be part of the advisory committee structure that will help it discharge its obligations under the Health and Safety at Work Act. One is the Advisory Committee on Toxic Substances, and will be chaired by the Director of the Health and Safety Executive's Hazardous Substances Division; committee members include four with relevant expertise and the CBI and TUC have each nominated four. Attention will focus on new chemicals, but not nuclear materials, which a new committee to replace the Nuclear Safety Advisory Committee will examine.

The other committee is the Advisory Committee on Dangerous Substances, to be chaired by the Executive's Deputy Director. This will concern itself with flammable and explosive substances, and includes three expert members as well as three each from the CBI and TUC.

A third committee, the Medical Advisory Committee, is already functioning, and one of the approximately 18 industry-based bodies, for agriculture, is also in operation.

Finland nuclear report

Finland should put a sharp brake on its nuclear energy plans, says the report of an energy committee under the leadership of the Finnish Minister for Trade and Industry, Arne Berner. Finland's shaky economic situation demands that investments should be confined to projects which will increase employment and the development of exports, rather than to nuclear power;

instead of the string of reactors which the government had thought of building around Finland's coast, the committee suggests that no new reactors should be built before 1985. If more energy is needed before then, it should come from non-nuclear sources and problems connected with nuclear energy should meanwhile be studied more closely.

Finland has four reactors under construction, at Lovisa and Olkiluoto, and the first is due to be loaded in a few months' time. By 1985 it is expected that they will be producing about 14% of the country's total energy consumption. Water power will supply about 23% (30% last year), with most energy continuing to come from oil, coal and peat.

There has been no general public debate about nuclear energy in Finland as there has been in Sweden, although groups living near the reactors have protested. The committee's report offers them no comfort. If Finland does build more nuclear power stations, it says, they will probably be sited near the existing ones.

Budgets announced

The 30-member Executive Board of the World Health Organisation last week recommended a budget for WHO activities in 1978 of \$165 million. The World Health Assembly will consider the proposal at its 30th session in Geneva in May.

The Council of the European Space Agency, meeting in Paris, approved programme budgets for 1977 last week of 481.1 million units of account, some 3 million down on those proposed. The programmes include Aerosat, Ariane, Marots, Meteostat, OTS and Spacelab. The Ministerial Council holds its first meeting later this month and will discuss the level of resources for 1978-80.

news and views

Self-control by bacteriophage lambda

from a Correspondent

It has been known for some time that the control of repressor synthesis in bacteriophage lambda is not a simple phenomenon. Such are the intricacies of the regulatory circuits involved in fact, that the subject was largely confined to European laboratories. Now, much of the lambda DNA sequence containing the *cis*-regulatory elements of repressor synthesis has been deciphered (Waltz *et al.*, *Nature* **262**, 665; 1976; Ptashne *et al.*, *Science* **194**, 156; 1976), and we can all take a crack at explaining the mechanism.

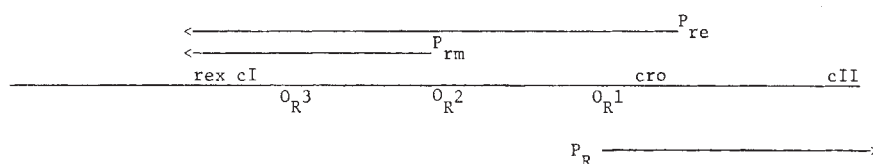
Shortly after infection of a sensitive *E. coli* by lambda, large quantities of repressor protein are synthesised. This repressor is translated from message initiating at a promoter, P_{rm} , located several hundred nucleotide pairs to the right of the repressor structural gene, *cI*. Transcription from P_{rm} depends on two phage products, *cII* and *cIII* which are not synthesised in an established lysogen. The maintenance level of repressor, about 200 repressor monomers per cell, is much lower than the infection level and depends on the activity of a different promoter, P_{ri} , located close to *cI*.

Transcription from P_{rm} , like transcription from P_{ri} , is not constitutive. Repressor can be inactivated and its rate of reappearance measured over a long period. What is found is that, once a cell has lost repressor, it is very slow to resynthesise it. Since a prophage makes but two phage products, repressor and Rex, it would appear that one of these is responsible for maintaining the activity of P_{rm} . The experiments of Waltz *et al.* and Ptashne *et al.* demonstrate that repressor is its own inducer.

By the use of restriction endonuclease DNA fragments containing the *cI* control elements, the control of repressor expression can be shown *in vitro* at the level of mRNA synthesis. If the fragment is incubated with RNA polymerase, no transcription of *cI* is observed. Instead, all the transcripts initiate at the P_{ri} promoter—the rightward promoter which is active in lytic phage development but repressed in a lysogen. Addition of a small amount of

repressor to the incubation mixture turns off P_{ri} transcription and activates *cI* transcription from P_{rm} . If large quantities of repressor are added, all transcription from the fragment is eliminated, including *cI*.

A simplified map of the control region is given below.



To the right of *cI* are three operators, or repressor binding sites, O_{R1} , O_{R2} and O_{R3} . These sites are 17 base pairs long and are separated by spacers 3 to 7 base pairs long. They differ slightly in sequence and function from each other; O_{R1} binds repressor more strongly than O_{R2} or O_{R3} . Sequences recognised by RNA polymerase for rightward transcription, P_{ri} , are located within O_{R1} and O_{R2} . Repressor, by binding to these operators, prevents RNA polymerase from binding to P_{ri} . O_{R3} is not required to repress P_{ri} ; its role is to regulate the synthesis of repressor in lysogens. The control region displays a symmetry centred around the middle of O_{R2} , such that O_{R3} contains a sequence similar to P_{ri} , but with the opposite orientation. This is P_{rm} .

The sequence of the control regions from mutant phage have been obtained and support these assignments. Mutations in O_{R1} or O_{R2} destroy their capacity to bind repressor. A mutation located between O_{R1} and O_{R2} inactivates P_{ri} , and a base change in the spacer between O_{R3} and O_{R2} destroys P_{rm} .

Speculating on the control of repressor synthesis on the basis of the DNA sequence we can see that the sequences of P_{rm} and P_{ri} are not quite identical. This could explain why an additional factor, repressor for example, is required for P_{rm} activation. Moreover, the P_{rm} -promoted mRNA begins with AUG, and the codon corresponding to the amino terminus of repressor is found immediately adjacent

to this triplet. Thus the P_{rm} message contains no leader sequence, hitherto thought to be essential for ribosome binding. This is not the case for the mRNA initiated at P_{ri} , which carries the sequence GGTGAT. Since, under optimal conditions, P_{rm} and P_{ri} are both very efficient promoters, the low

level of repressor in a lysogen is due to inefficient translation of the P_{rm} mRNA, presumably because it is leaderless.

Unfortunately, it is still not obvious how repressor stimulates *cI* transcription. Perhaps P_{rm} competes with P_{ri} for RNA polymerase, and the role of repressor is to block the binding of RNA polymerase at P_{ri} . If this were the case, we would expect that mutations which reduce the efficiency of P_{ri} as a promoter would relieve the repressor requirement of P_{rm} . Mutations isolated so far in P_{ri} do not have this character. Alternatively, repressor bound at O_{R1} may interact with RNA polymerase to stimulate directly its binding at P_{rm} . Ptashne notes that the distance between O_{R1} and the initiation of *cI* transcription is the same as the distance between the start point of *lacZ* transcription and the site in the *lac* operon where the positive-control cyclic AMP binding protein interacts.

With either model, repressor synthesis in a lysogen will be negatively regulated at O_{R3} , where repressor protein competes with RNA polymerase for binding to DNA. The interplay of these positive and negative control circuits assures that the maintenance level of repressor will be confined to narrow limits.

The ability to isolate and sequence mutations in regulatory regions is certain to be tested again at other interesting lambda loci; notably at P_{re} and at the sites of action and recognition of the lambda control functions *N*, *Q*, *cII*, and *cIII*. □

Triassic extinction or Jurassic vacuum?

from Andrew Milner

IN Charles Kingsley's *The Water Babies*, the author makes the observation that one cannot claim that water babies do not exist until one has seen them not existing. This little paradox is particularly pertinent to the fossils of terrestrial animals and what we deduce about them, particularly when they are absent from rocks of a given age.

If a taxon is absent from the fossil record for a given period of time, we logically assume that either it was genuinely absent or that it was present but that circumstances did not permit preservation. If the taxon or its descendants subsequently reappear as later fossils or living forms, we can be certain that the absence was due to non-preservation. If it never reappears the problem remains open and becomes: when did this taxon become extinct? Attributing a point in time to an extinction is only valid when the fossil record is comparably complete before and after the supposed event. Only if an animal is absent from an otherwise rich assemblage or if its ecological replacement is present, can one claim to have seen that animal "not existing".

The discovery of a temnospondyl amphibian in the Lower Jurassic of Australia announced by Warren in this week's issue of *Nature* (page 435) is a reminder of this. The Order Temnospondyli radiated during the Carboniferous to reach its greatest diversity in the Upper Carboniferous and Lower Permian. Many lineages became extinct during the Permian but others extended into and through the Triassic, and by the late Triassic several families remained, including the capitosaur, brachyopids, metoposaurs and plagiosaurs. That the extinction of these families had taken place at the end of the Upper Triassic was virtually dogma. So much so that, as Warren notes, an earlier discovery of a fragment of Jurassic temnospondyl had been assumed to be a reworked bone from the Triassic simply because there was no precedent for it to have been any younger.

However, in order to have such fossils of Lower or Middle Jurassic amphibians or terrestrial vertebrates, one must first have vertebrate-bearing continental sediments of the same age and these are singularly scarce. The Upper Triassic of Europe, North America, Africa, India and China has produced an extensive range of terrestrial vertebrates including temnospondyl amphibians, procolophonids, chelonians, thecodonts, rhynchosaurs, dicynodonts,

and early dinosaurs, crocodiles and mammals. By the Upper Jurassic, a very different assemblage of continental vertebrates is known from Europe, North America and East Africa. The chelonians, crocodiles, dinosaurs, pterosaurs and mammals predominate. The temnospondyls, procolophonids, rhynchosaurs, thecodonts and dicynodonts are all absent, presumed extinct, and in most cases their ecological replacements can be seen to be present. But when were they replaced? Did they become extinct synchronously on the Triassic-Jurassic boundary as a literal interpretation of the record would suggest? Or were they gradually replaced during the Lower and Middle Jurassic? Warren's amphibian suggests the latter possibility as do the tritylodonts which extend from Upper Triassic to Middle Jurassic.

Several recent discoveries offer the hope that this problem will eventually be solved. A sauropod dinosaur has recently been described from the Lower Jurassic Kota formation of India (Jain *et al.*, *Proc. R. Soc.* **B188**, 211; 1975) and new microvertebrate localities of Middle Jurassic age have been found on the Isle of Skye (Waldman and Savage, *J. geol. Soc.* **128**, 119; 1972) and in Oxfordshire (Freeman, *Science* **194**, 1053; 1976). We still do not have a rich assemblage of early Jurassic terrestrial vertebrates but further excavation of these localities and Warren's Queensland locality may provide us with enough material to characterise the Lower and Middle Jurassic faunas and hence determine the chronology of the turnover of terrestrial vertebrate taxa between the Upper Triassic and Upper Jurassic. □

Astrophysics in Boston

from James Pringle

The Eighth Texas Symposium on Relativistic Astrophysics took place on December 13-17, 1976 in the Copley Plaza Hotel in Boston, Massachusetts. The proceedings of the Symposium will be published as a special issue of the *Annals of the New York Academy of Sciences*. Nearly a thousand astronomers and physicists were present for this biennial jamboree and there might have been many more but for the competing attraction of the mid-January American Astronomical Society which was taking place in Hawaii.

X-RAY astronomy featured prominently in four of the ten sessions. In the session on X-ray astronomy itself R. Novick (Columbia University) reported on the first unambiguous detection of polarised radiation in X-ray astronomy. With the X-ray polarimeter on board OSO 8, he and his group have found that at X-ray energies the Crab Nebula is 22% polarised with a significance of some 15 standard deviations. This compares with 19% polarisation in the optical. The position angle of the polarisation is essentially the same in both energy bands. He presented marginal evidence for the polarisation of the X rays from the Crab pulsar itself, and indications that the swing in position angle of the X-ray polarisation

direction through each pulse is comparable to that seen in the optical. In addition Cygnus X-1 may be polarised at the 3% level (a $2\frac{1}{2}$ standard deviation result) and, at a higher level of confidence, Cygnus X-2 may be polarised at the 5% level. E. Boldt (Goddard Space Flight Center) reported on some further high time resolution observations of the black hole candidate X-ray source Cygnus X-1, which continues to display X-ray activity on timescales as short as a few milliseconds. He gave tentative evidence that there may be a repetitive occurrence in these millisecond bursts with a timescale of about 10 ms and speculated that this might be identified with material falling within the last stable circular orbit for a black hole of some 17 solar masses.

Ken Pounds (University of Leicester) gave a preliminary report on the catalogue of X-ray sources being prepared from data from the Ariel V satellite and on a programme of optical identification of previously unidentified high galactic latitude sources (UHGLs for short). He argued that most of the 50 or so UHGLs in the UHURU catalogue of X-ray sources are now accounted for and so the UHGL mystery is solved, although the attentive in the audience noted that he had at the onset reported some 45 new UHGLs found by Ariel V.

The discovery of bursting behaviour

at X-ray energies is becoming increasingly common, although the implied link in the title of the session "X-ray bursters and Globular Clusters" was rather controversial. Walter Lewin (Massachusetts Institute of Technology) argued strongly that, although two of the bursting sources were almost certainly within globular clusters, the connection between the others and globular clusters was by no means proven and that the distribution of bursters within the galaxy is unlike that of the globular clusters. Jerry Ostriker (Princeton University) disagreed and argued, perhaps less convincingly but no less strongly, that the two distributions are remarkably similar. Lewin went on to discuss individual bursting sources of extraordinary complexity from MXB 1743-29 which bursts only once a day or so to MXB 1728-34 which is known as the 'rapid burster' producing bursts of X rays some 5,000 times a day. As more and more details of the bursting behaviour accumulate, it becomes more difficult for theorists to find a convincing explanation of the phenomena. Don Lamb (University of Illinois) was given the problem of finding something intelligent to say on the theoretical aspects of the bewildering observational results. He discussed a variety of possibilities including bursts of nuclear burning on the surfaces of neutron stars, and accretion events involving material held up by the magnetosphere of a neutron star sporadically going unstable and crashing to the surface.

Part of the limelight at the session on Neutron Stars was stolen by J. Trümper (Max-Planck-Institut, Munich) who had flown in especially to announce data obtained in a balloon flight looking at Hercules X-1 in the energy range 20-120 keV. It had been thought previously that the X-ray spectrum of Hercules X-1 did not extend significantly above about 30 keV. Trümper reported that the source had been seen well above 30 keV and the X-ray pulses (with a period of 1.24 s) were seen up to energies as high as 70 keV. Moreover, he presented evidence for an emission feature in the spectrum above 40 keV which he interpreted as an emission line due to cyclotron radiation at an energy of 53 keV—there is no other likely reason for the production of an emission line at this energy. If this is true, it implies that Hercules X-1 is indeed a magnetised neutron star and that the magnetic field is in the region of $2-5 \times 10^{12}$ gauss (depending primarily on which harmonic of the gyrofrequency the emission is supposedly produced). Following this talk, Leventhal (Bell Laboratories) reported on γ -ray balloon observations of the Crab Nebula made

Membrane-bound proteins

from I. B. Holland and R. H. Pritchard

MEMBRANE-ASSOCIATED proteins are hard to study. They will probably be insoluble in aqueous solvents, there is often no simple assay for them and consequently they will probably defy purification. Studying their function may not be a simple matter either. The way a protein which facilitates transport of a small molecule across a membrane works will need to be investigated with the protein *in situ* or attached to an artificial membrane. Silhavy *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3423; 1976) have found a new way of studying these proteins using the sophisticated box of tricks available to the *Escherichia coli* geneticist. They selected rare variants of this bacterium in which a gene coding for a protein involved in transport of maltose, and thought to be membrane bound, was fused to a gene coding for an easily assayable enzyme (β -galactosidase). The fused genes made a fusion protein which had the NH_2 -terminal end of the transport protein

and the COOH-terminal end of β -galactosidase. This hybrid protein had β -galactosidase activity which could be used to show that it was located in the cytoplasmic membrane. Thus an enzyme normally found in the cytoplasm had been carried to the membrane suggesting that the maltose transport protein is indeed membrane bound and that its COOH-terminus is not needed for this binding to occur.

Silhavy *et al.* hope to use their technique to investigate the structural features of proteins which lead them to associate with membranes. Their experimental strategy may be equally useful in identifying otherwise non-assayable proteins and for histochemical studies.

Unfortunately, in organisms with underdeveloped or emerging genetic technologies it will not be possible to engineer the variants required to make fusion proteins. This may only be a temporary difficulty in the exploitation of a promising idea.

to detect γ -ray lines. He gave evidence for a strong γ -ray line at an energy of 400 keV and speculated that this might be the 510 keV electron-positron annihilation line redshifted from the surface of the Crab pulsar: the redshift would imply that the pulsar is a neutron star of mass 1.4 solar masses, a value highly favoured on stellar evolutionary grounds.

Low mass X-ray binaries were discussed by Anne Cowley (University of Michigan) in the session on Close Binary Stars. The most intriguing source recently discovered to emit X rays is the star AM Herculis. This was already known to be a "nova-like variable", implying that a large amount of flickering is present in the optical light. It has since been found to display a 3-h period, probably due to orbital motion, in optical light and in radial velocity and to emit soft X rays which vary with the same period. Most remarkably, the optical light is highly circularly polarised (5-10%) with the degree of polarisation changing on the same 3-h period. It seems that this polarised light can only be due to cyclotron emission, implying the presence in the system of a magnetic field with a strength of over 10^8 gauss.

The second morning was devoted to quantum theory in strong gravitational fields. Stephen Hawking (Cambridge University) spoke on black holes and unpredictability. Inside a black hole, there is always a singularity which is a

place where space and time come to an end and all the laws of physics break down. Provided the black hole can prevent any information about the singularity from leaking out, however, any observer not in a black hole would not be affected by the breakdown of predictability which occurs at the singularity. This "cosmic censorship" is endangered by Hawking's discovery that quantum effects allow particles and radiation to tunnel out of black holes. This radiation has an added degree of randomness over and above that associated with quantum mechanics. Equivalently, he concluded, one could say that "new random information is entering our universe from regions (either singularities or 'worm holes' leading to other universes) about which we have no knowledge".

In the session on Gravitational Theories Larry Smarr (Harvard University) gave a talk with the ominous sounding title "Computer Generated Space-Times". He reported on a continuing effort to solve Einstein's field equations in their full time-dependent form by means of numerical methods. One of the aims of this research would be, for example, to study the collision of two black holes with a view to discovering how much energy is radiated away in the form of gravitational radiation.

Irwin Shapiro (Massachusetts Institute of Technology) opened the session on quasars with a talk entitled

"Apparent Superrelativistic Expansion of Quasars: Evolution or Revolution?". He reported on further radio VLBI (very long baseline interferometry—with baselines extending across the Atlantic) observations of quasars which show source structure down to an angular size of 0.001 arc s or less. Almost all the sources observed show time variations and some 10–15% of them show components which are separating from each other with apparent velocities greater than that of light. In the quasar 3C279 two compact radio sources are seen about 8 parsecs apart which appear to be flying apart at a speed some 25 times that of the velocity of light. There is as yet no convincing explanation of this phenomenon.

Dealing with theories of quasars, Martin Rees (Cambridge University) argued strongly that the observational evidence, including the VLBI observations presented by Shapiro, all tended to indicate that quasars are powered by a single massive object in the nucleus of a young and distant galaxy, and further that the most likely candidate for such an object would be a massive black hole. He described how such a black hole could provide the huge luminosity required (about 10^{46} erg s⁻¹) by slowly devouring the dense cluster of stars that is likely to be around it.

The final afternoon session was devoted to a discussion of galaxies. Harry van der Laan (Leiden Observatory) presented the latest observations of double radio galaxies by the Westerbork telescope. Polarisation observations provide a means of delineating the global structure of the magnetic field in the giant radio lobes: in general the field lies along any elongated features extending over very large distances and lies perpendicular to the intense radio hot spots at the outer edges of the radio lobes, as if the spots act like snow ploughs gathering field as they expand from the centre. He also discussed statistical evidence on the way the power houses which produce the double radio sources are distributed among elliptical galaxies. Somewhat surprisingly the evidence suggests that although the more massive (and brighter) elliptical galaxies are more likely to be endowed with an active power house, the size of the power house provided is independent of the size of the galaxy in which it is placed.

E. Salpeter (Cornell University) presented some 21-cm observations of galactic rotation curves using the newly re-surfaced Arecibo disk to extend the measurements out to radii of some 100 kpc from the centre. The graph of rotation velocity against radius should remain roughly constant if there is a

massive unseen halo of faint stars (or even Jupiters) surrounding the optical galaxy, but should otherwise decrease with increasing radius. He found that the only galaxies observed which did not show evidence for a massive halo were those which had a close enough binary companion which would by now have stripped away the outer parts of any such halo, had it ever existed.

Alar Toomre (Massachusetts Institute of Technology) ended the conference with a light-hearted and amusing talk showing how tidal interactions between galaxies can produce spiral structure tantalisingly similar to that observed. He finished by showing how if a perturbing galaxy scores a "bullseye" by passing through another galaxy sufficiently close to the centre, the perturbed galaxy shows a bright, expanding ring structure. □

Animal behaviour and earthquake prediction

from John M. Logan

A conference on Animal Behaviour and Earthquake Prediction, sponsored by the United States Geological Survey, was held in Menlo Park, California on October 23–24, 1976.

CAN variations in animal behaviour help to predict earthquakes? As early as 100 AD Pliny advocated its use, but until recently the topic has received little serious scientific attention. Efforts now being made by the Chinese have, however, stimulated renewed interest in the topic. It has become especially relevant as other postulated precursors such as v_p/v_s ratios, electrical resistivity, radon content, electromagnetic variations and changes in the electrostatic field are either being questioned or are in the developmental stages.

The first question to be asked is whether there is any scientific basis for the popular reports of abnormal behaviour of animals, birds, fish and reptiles before earthquakes? If so, can animal behaviour then be considered as a reliable predictive indicator; what are the physical or chemical factors causing the anomalous behaviour; what appropriate research programme can be suggested to investigate behaviour patterns and their associated stimuli; and finally, can the biological indicators eventually be replaced by physical receivers? It became apparent at the outset that the various skills of geologists, geophysicists, and biologists

would be needed. Thus, possibly the most diverse group of scientists ever to discuss the problem of earthquake prediction was brought together at the conference.

Anecdotal reports by non-scientists from many parts of the world contain many similar observations of animal behaviour before earthquakes. These observations come from people who could not possibly have had contact with each other or have read reports from other areas. This lends credibility to the observations, and provides a large body of evidence supporting the contention that animals do sense something before earthquakes. Apparently the response of animals is not restricted to a single species or genus, but spans a very wide spectrum of biological forms which suggests that there is either more than one stimulus or a universal one that can be detected by a wide variety of organisms. Furthermore, with the possible exception of reports of snakes coming out of the ground in mid-winter, few of the responses reported are truly 'abnormal' or extreme. Most involve normal forms of activity which take place either at elevated levels or at unique times. Thus, the term "abnormal behaviour" is somewhat misleading.

D. Mead (San Jose Mercury) witnessed the unusual behaviour of a cow immediately preceding one of the aftershocks at Oroville, California, and was told that similar actions had been observed before previous earthquakes. Soon he felt the aftershock. Most convincing of all was the report by H. Kraemer, B. Smith, and S. Levine (Stanford University) on the behaviour of chimpanzees preceding a magnitude 3.7 event. This is one of the few scientifically based studies available, and the correlation between the chimpanzees' behaviour patterns and the earthquakes is very convincing.

Precursive physical phenomena associated with earthquakes that might serve as stimuli for recognisable variations in animal behaviour were discussed at some length. The important point was made that earthquakes have a number of causal mechanisms rather than any single one. Fracture of rock along a new fault, breaking of asperities along existing fault zones, fracture of gouge zones, material and other instabilities along fault surfaces all may release seismic energy. It is possible that not all earthquakes are preceded by the same precursive events, and it follows that potential stimuli for perturbations in animal behaviour may not be present in all earthquakes.

It was pointed out that observed changes in air pressure, gravity, elevation and tilt all fall within the normal range experienced by animals throughout the day and are doubtful stimuli of

behavioural variation. D. Hill (US Geological Survey) demonstrated that acoustic emissions could only be considered a potential stimulus if they were centred within a few hundred feet of the surface and almost beneath the animal. Observed variations of a few gammas in electromagnetic signals associated with earthquakes also seem unlikely to produce significant variations in biological responses, as these are an order of magnitude less than the normal diurnal variations experienced by the animal if it stands still; if it moves about, it will experience changes of hundreds of thousands of gammas in a single day. Changes in near surface water level may provide a possible explanation for some behaviour such as snakes leaving their holes in mid-winter, but such changes certainly cannot be cited as a stimulus for the majority of the reported behavioural perturbations. The two remaining areas—changes in the electrostatic field and gas emissions before earthquakes—have not been investigated in great detail and both may be potential stimuli. The conclusion reached was that either electrostatic (or other electrical) effects are the primary causes of unusual animal behaviour, or some other effect not yet investigated (smell?) is operating.

A number of examples of the extreme sensitivity of biological systems to small changes in their environment was reported. Most of the anecdotal reports of animal behaviour can probably be classified as startle responses and are probably not learned. Stimuli producing the startle response may simply exceed some noise level or may be due to variations of the pattern of stimuli within the normal range experienced by the animal. If the latter is true, then detecting variations in patterns of stimuli will prove much more difficult than detecting a critical level of the signal.

In conclusion, it was pointed out that an earthquake prediction programme making use of animal behaviour may take two forms. The first is the recognition of variations in animal behaviour preceding earthquakes and its use as a predictive tool without understanding the mechanics of the process. This empirical approach, if successfully developed would satisfy the immediate need of earthquake prediction. Research could, and probably should, be oriented to this end. The second approach involves an investigation of the stimuli involved, which could lead to direct monitoring of the physical signal without the intermediate step of observing animal behaviour.

The interdisciplinary nature of the problem is extremely stimulating. Future research will clearly involve

geologists, geophysicists, and biologists. I feel that the conference has successfully identified the problem, asked the pertinent questions and posed a number of hypotheses. Future work, it is hoped, will bring this to a level of scientific benefit to mankind. □

Moon measurements

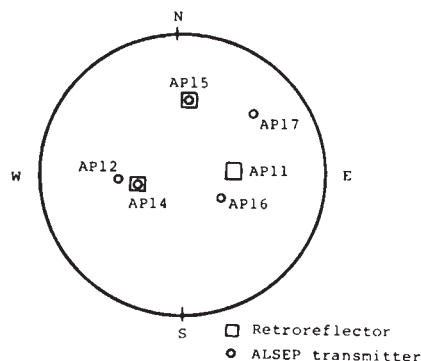
from A. H. Cook

PLACING optical retro-reflectors and radio transmitters on the surface of the Moon has proved to be one of the most significant and lasting achievements of the Apollo programme. The retro-reflectors of which three were placed in the US programme and one (of French construction) in the USSR programme, have been used to reflect pulses of light emitted by laser transmitters on the Earth so that the round trip travel time of the pulse and hence the distance between a point on the Earth and one on the Moon can be measured very accurately, to within about 30 cm at present. So far almost all the measurements have been made from the Macdonald Observatory in Texas (Bender *et al.*, *Science* **182**, 229; 1973). The radio transmitters have been observed by interferometers with very long baselines (VLBI) which are sensitive to the rotation of the group of transmitters about the line joining the centres of the Earth and the Moon (King *et al.*, *J. geophys. Res.* **81**, 6251; 1976). Accuracies corresponding to between 1 and 10 m on the surface of the Moon are achieved.

The distance between a point on the Earth and one on the Moon depends on three factors: the motion of the Moon in her orbit about the Earth, the rotation of the Earth upon its axis and the oscillations of the Moon about her centre of mass. The three factors can

be separated (the separation would be sharper if more laser observatories were in operation on the Earth and especially one in the southern hemisphere) and in consequence a great deal of physics can be studied. Thus the orbit of the Moon contracts because of the friction which damps the tides upon the rotating Earth; it may also be possible to check certain rather general relativistic theories. It is hoped that precise measurements of the relative positions of terrestrial observatories will lead to direct observation of movements of tectonic plates and to improved understanding of the very imperfectly known variations in the vector of the Earth's spin. In astronomy improved measurements of the Moon may lead to a better fundamental catalogue of star positions.

One of the most exciting consequences of the observations is the improved knowledge of the lunar librations. The Moon moves about the Earth in such a way that, seen from the Moon, the Earth remains almost but not quite in a fixed position relative to the axes of inertia of the Moon. Now the Moon has three unequal moments of inertia and also there are important terms of high harmonic order in the gravity field, and in consequence when the Earth moves with respect to the Moon it exerts torques which cause the Moon to rock about the lunar centre of mass. That rocking motion is the physical libration of the Moon. The amplitudes are small and difficult to observe visually from the Earth but by using laser ranging to the Moon the motion has already been observed in detail with high accuracy. The interest of observations of the libration lies in the fact that the libration depends on the moments of inertia of the Moon and when the observations of libration are combined with those of the second harmonic coefficients of gravity as obtained from artificial satellites about the Moon (Gapcynski *et al.*, *Geophys. Res. Lett.* **2**, 353; 1975), then the three moments of inertia may all be obtained separately (Williams *et al.*, *The Moon* **8**, 469; 1973). The moment of inertia of a celestial body is a key property in the study of its constitution because it depends on the way the density varies from the centre outwards. If the density were constant the moment of inertia in non-dimensional form would be 0.4 exactly, and when the density decreases towards the surface, the non-dimensional moment of inertia is less. The value for the Moon now seems to be close to 0.392, less, but not much less than 0.4. This is a stringent constraint on models of the lunar interior and because the value is so close to 0.4, it is most desirable to have it measured with as great an accuracy as possible.



Retro-reflectors and radio transmitters used for analyses of lunar librations (King *et al.*, *op. cit.*). AP denotes the Apollo mission which carried the equipment.

It is here that the radio transmitters come in. Because of the fact that the technique of VLBI measures the rotation of the group of transmitters about the line of centres of the Earth and the Moon, it is insensitive to the Earth's rotation and to the Moon's orbital motion, and so leads to a very clear separation of the librations from other motions. The publication (King *et al.*, *op. cit.*) of the results obtained by combining lunar laser ranging with VLBI observations of the lunar radio transmitters is thus a landmark in lunar studies. The remarkably low uncertainty of 0.003 is attributed to the estimate of 0.392 for the value of the non-dimensional polar moment of inertia. It is also important that certain of the higher gravitational harmonics can be derived from the librations because there are considerable uncertainties in their derivation from the orbits of artificial satellites alone.

Clearly a great advance has been made in our knowledge of lunar dynamics in the past few years, and that in itself emphasises how much there is still to learn about the dynamics of the Earth. As already mentioned, one of the most important steps forward would be to obtain data from a lunar laser ranging observatory in the Southern Hemisphere, and there are indeed now good prospects of obtaining such data as a result of co-operation between US and Australian groups, in which British workers are also participating. As ever when new possibilities of precise measurements are realised, a new world of knowledge seems to be opening. □

Cheap uranium from bacteria?

from a Correspondent

An International Atomic Energy Agency research coordination meeting on the bacterial leaching of uranium ores was held at the University of Warwick on December 13-15, 1976 under the chairmanship of Professor Donovan Kelly, of the Department of Environmental Sciences, University of Warwick. The meeting was formally hosted by the UK Department of Energy.

DURING the 1960s it was found that uranium could be recovered from the acidic waters running through dumps of uranium mining wastes or from water hosed on to the walls of worked-out uranium mines. This solubilisation

of uranium was subsequently found to be due to the activity of a specialised group of unusual bacteria called *Thiobacillus ferrooxidans*, which obtain energy for their growth from oxidising iron from the ferrous to ferric form in very acidic environments. These bacteria are always found in acid mine waters when pyrite (iron sulphide) is present in the ore being mined. They attack the pyrite and produce sulphuric acid and ferric iron, which in turn chemically converts the insoluble uranium ore into soluble uranyl sulphate. The possible economic importance of this process as a potentially cheap means of uranium recovery was early recognised by the IAEA, whose headquarters are in Vienna, and which has supported research and development into the bacterial leaching of uranium for a number of years.

The meeting at Warwick University was the third coordination meeting (earlier ones were in Ankara in 1972 and Vienna in 1974), held to review recent developments and to review the present status of the whole subject after some ten years of active work in many countries. Scientists from Austria, Canada, Finland, Hungary, India, Romania, South Korea, Yugoslavia and the UK and USA took part. Much of the work discussed had been financed by the IAEA and ranged from the basic microbiology of the process over economic aspects to the safety and control of uranium recovery.

With the likely demand for uranium for nuclear power generation in the Western World alone between 1976-2000 standing at 4 million tonnes of U_3O_8 , mining companies have increasingly been looking at ways of recovering uranium from ores containing progressively less uranium, and to ways of recovering the small amounts of uranium left in the wastes from the processing of richer ores. Bacterial leaching provides just such a process, since the bacterial activity is as effective in removing essentially all the uranium from a 0.03% U_3O_8 ore as from a 0.3% ore, without necessarily being more costly, whereas the difference between these ores for conventional extraction processes is sufficient for the lower grade one to be uneconomic to work at acceptable prices. Bacterial leaching thus enables countries with only low-grade 'uneconomic' ores to exploit their natural resources cheaply and of course can also be used for low cost recovery of uranium from high-grade ores.

Since the IAEA programme began, the state of the art of bacterial leaching has progressed from minimal understanding and exploitation to the current state of having a rather full understanding of the fundamental

microbiology and biochemistry of the process, to have proved large scale pilot plants and built at least one commercial scale plant. The latter, reported at the meeting, will become operational in Canada in 1977 and will produce a million pounds of U_3O_8 a year, using 3,000 tonnes of crushed ore per day.

The meeting discussed the basic studies of growth and physiology of the iron bacteria, largely carried out by Professor Kelly's group, the immunological characterisation studies performed in Korea, and work on the control of leaching activity and pilot plant operation in Romania, Yugoslavia and India. Much time was devoted to the evaluation of large scale plants, their engineering design, efficiency, responses to environmental factors such as seasonal temperature variation, supply of oxygen and of other nutrients, and possible hazards in the operation of the extraction systems. Britain has been active in the design and evaluation of practical leaching systems and the use of the bacteria to generate ferric iron from various sources to be used in leaching. A report on the work of the Warren Spring Laboratory in this field was received with considerable interest. The very large scale alkaline leaching of uranium in Hungary was reported in full and provided further information of basic use in plant design.

The conclusions of the meeting, reported to the IAEA, included the recommendation that bacterial leaching of uranium should be developed and encouraged as a cost-competitive method of recovering uranium, involving smaller capital and running costs than conventional methods, with the incalculably valuable bonus of enabling treatment of ores otherwise prohibitively costly to treat. It was foreseen that work in this field would proceed to progressively more controlled processes and that the areas requiring further work could be detected by constructing the realistic process flow sheets and mathematical models that work to date had made feasible.

A long-standing problem in many mining operations is the release of polluting acids and metals into the environment. One conclusion of this meeting was that controlled exploitation of leaching of waste rock from conventional mining operations was to be encouraged and exploited not only to recover uranium and other metals, but to minimise the likelihood of environmental pollution by uncontrolled natural leaching. It is clear that the economic, biological, sociological and engineering aspects of the bacterial recovery of uranium will occupy many workers for years to come. □

review article

Targeting of drugs

Gregory Gregoriadis*

Recent work suggests that specific association of drugs with their targets could be achieved by means of a versatile carrier capable of transporting drug molecules from the site of application directly to the site of action.

AN important prerequisite for success in the application of pharmacologically active agents is specificity. Unfortunately, most agents interact with non-target sites as well. This is well illustrated in cancer chemotherapy of which the slow progress can be attributed to the failure of cytotoxic molecules to act on malignant cells only. Poor selectivity can often be worsened by the failure of agents to enter the target area. Thus, in certain cases of drug resistance development, cells (for example malignant cells) become impermeable to drugs, and in antimicrobial therapy antibiotics have little or no access to intracellular sites harbouring microorganisms. Access of drugs to the target can also be prevented because of gross anatomical barriers. Penetration of the blood-brain barrier by enzymes for instance would be desirable in the treatment of some lysosomal storage diseases¹.

There are at least two options open to us in pursuing optimal drug action. (By drugs I mean not only conventional drugs, enzymes and proteins used in medicine, but also substances used in biological research.) In the conventional one, efforts are directed towards the creation of specialised molecules. Usually a therapeutically profitable target-agent relationship is far from ideal, and undesirable side effects are almost always present. The alternative could prove simpler: functional molecules, not necessarily target-specific, could be transported by a carrier to the site of action and released to perform their task. Transport should be effected in isolation from the biological space between the site of application and the site of action because this might be important in where agents are either prone to inactivation or detrimental to the non-target space in the host. The carrier itself should be non-toxic, biodegradable and of the appropriate shape and size to accommodate a wide variety of substances. It should preferably ignore, or be ignored by, irrelevant areas and have a pronounced affinity for, and easy access to, the target site within which there should be a mechanism for the release of agents from the carrier. The latter, having accomplished its function, would then be disposed of.

In searching for the ideal carrier, one is tempted to look for systems which can intrinsically 'recognise' the target (Table 1). Thus, antitumour drugs complexed to antibodies have been shown to improve cytotoxicity to malignant cells possessing antigens corresponding to the immunoglobulin carrier². As binding to the immunological determinants of the membrane is believed^{30,31} to initiate endocytosis, the action of the antibody-bound drug is likely to be lysosomo-

tropic (Fig. 1b). In spite of its appeal the antibody carrier approach is associated with a number of difficulties among which the isolation of target specific antigens for the production of antibodies and the purification from these of immunoglobulin molecules specific for the antigen, are prominent. In addition, drugs attached to immunoglobulins may mask³³ the antigen recognition site of the protein or alter its tertiary structure and render it prone to rapid removal by the reticuloendothelial system. Assuming that target-carrier contact can be made one may ask whether the immunoglobulin-drug complex will allow the active moiety (drug) to perform its task. Severance of the bond (usually an ester) between carrier and agent may be necessary and as there could be a variety of linkages to suit a variety of agents, there should be a corresponding number of esterases. Other difficulties include inhibition of the immunoglobulin-antigen (target) interaction by circulating antibodies raised by the host against the antigen and also the possibility of allergic reactions to the carrier itself.

An interesting group of macromolecular carriers, only modestly pursued, is asialoglycoproteins (Table 1). These exhibit *in vivo* a highly specific affinity for the hepatic parenchymal cells³⁴ by which they are taken up into the lysosomal apparatus³⁵. Using asialofetuin it was possible¹⁰ to direct to the liver lysozyme and albumin cross-linked to the protein, and a similar use could perhaps be made of agalactoglycoproteins and hexosamine glycoproteins which possess specificity for the liver and kidney respectively³⁶. Other potential carrier molecules are trophic hormones, lectins³² and some lysosomal enzymes³⁷. In contrast to antibodies, many of these substances are readily available and complications in target recognition are less likely to occur *in vivo*. However, they lack the breadth of target choice of immunoglobulins as a group.

In a different approach for target selectivity advantage is taken of the ability of a number of cell types (for example phagocytes and certain malignant cells) actively to endocytose macromolecules. Among the latter (Table 1) a notable example¹⁵ is DNA, to which cytotoxic drugs such as daunomycin and adriamycin bind tightly upon contact. Experiments with DNA-bound daunomycin and adriamycin have shown that reduced toxicity³⁸ of the bound drug and, presumably, increased uptake of the DNA-drug complex by tumour cells contribute to cures of tumour-bearing animals^{15,17} and to remissions of certain malignancies in man¹⁸. In another example¹¹, β -amanitin coupled to albumin was found to induce *in vivo* selective damage of the sinusoidal liver cells and kidney proximal tubule cells, both very active in the endocytic uptake of proteins. As with most macromolecules discussed, these lysosomotropic (Fig. 1) carriers can be vulnerable to the biological milieu (for example

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Table 1 Drug carriers in biology and medicine

Carrier	Associated drugs
Macromolecules	
Immunoglobulins ²	Methotrexate ³ , daunomycin ⁴ , adriamycin ⁴ , chlorambucil ^{5,6} , radioactive iodine ⁷ , diphtheria toxin ⁸ , glucose oxidase ⁹
Desialylated glycoproteins	Lysozyme ¹⁰ , albumin ¹⁰
Albumin	Amanitin ¹¹ , phalloidin ¹² , deoxycholic acid ¹³
Fibrinogen	Dichloroethylphosphoramidate ¹⁴
Deoxyribonucleic acid	Daunomycin ^{15,16} , adriamycin ¹⁷
Dextran	Insulin ¹⁸ , noradrenaline ¹⁸ , amphetamine ¹⁸ , novocaine ¹⁸ , coenzyme B ₁₂ ¹⁸
Cells	
Erythrocytes	β -glucosidase ¹⁹ , β -galactosidase ¹⁹ , glucuronidase ²⁰ , asparaginase ²¹ , glucose oxidase ²² , methotrexate ²³ , adriamycin ²³
Leucocytes	'Corrective' enzyme ²⁴
Hepatocytes	Uridine diphosphate glucuronyltransferase ²⁵
Synthetic systems—non-biodegradable²⁶	
Nylon semi-permeable microcapsules	Carbonic anhydrase ²⁶ , uricase ²⁶ , asparaginase ²⁶ , urease ²⁶ , catalase ²⁶
Polyacrylamide gel	Asparaginase ²⁷
Liquid surfactant membranes	Urease ²⁸
Glass beads	Uricase ²⁹
Synthetic systems—biodegradable	
Albumin microspheres	6-mercaptopurine ³⁰ , actinomycin D ³¹
Multiple oil emulsions	Naltrexone ³²
Lactic acid polymers ³³	Cyclazocine ³³
Ufasomes (unsaturated fatty acid spheres) ³⁴	
Liposomes ³⁵	Lysozyme ³⁶ , amyloglucosidase ^{37,38} , neuraminidase ^{39,40} , α -mannosidase ⁴¹ , glucocerebrosidase: β -glucosidase ⁴² , hexosaminidase A ⁴³ , asparaginase ⁴⁴ , peroxidase ⁴⁵⁻⁴⁷ , glucose oxidase ⁴⁸ , lectins ⁴⁹ , immunoglobulins ^{46,50-53} , insulin ^{54,55} , diphtheria toxoid ⁵⁶ , Poly(I):Poly(C) ⁵⁷ , actinomycin D ⁵⁸⁻⁶¹ , 5-fluorouracil ⁶² , methotrexate ^{63,64} , bleomycin ^{18,50,65} , cytosine arabinoside ⁶⁶ , 8-azaguanine ⁶⁷ , mechlorethamine ⁶⁸ , bichloroethyl nitrosourea ⁶⁸ , colchicine ⁶⁹ , penicillin G ⁷⁰ , steroids ^{71,72} , cyclic AMP ⁷³ , pepstatin ⁷⁴ , glutamate ⁷⁵ , nitroblue tetrazolium ⁷⁶ , EDTA ⁷⁷ , DTPA ⁷⁷ , TcO ₄ ^{78,79}

Association of drugs with carriers can be effected through covalent, hydrophobic, hydrogen or other types of bonding. In synthetic systems passive entrapment of drugs is also possible. Only representative bibliography is shown for antibody², non-biodegradable²⁶ and liposomal³⁵ carriers. For more information see respective references.

blood nucleases in the case of DNA) and are limited in both range of agent accommodation and target choice.

A number of three-dimensional systems (Table 1) have been proposed as alternatives to the 'linear' macromolecular carriers. Depending on their composition, such systems can be biodegradable or non-degradable. Of the latter, a well-known example is the nylon semipermeable microsphere²⁶ within which various enzymes have been entrapped and found capable of acting on inwards diffusing substrates. Like most non-biodegradable carriers, however, these are unsuitable for parenteral (chronic) use. Of the biodegradable systems on the other hand, whole cells could prove of considerable value. Erythrocytes (Table 1) for instance could be useful in delivering agents to the reticuloendothelial system¹⁹ into which these cells eventually find their way. Furthermore, and assuming that their life span *in vivo* is largely unchanged by the procedure of agent entrapment, erythrocytes could serve as devices for the slow release of drugs into the circulation. Alternatively, undesirable substrates could enter erythrocytes to be degraded by entrapped enzymes²¹. While erythrocytes are limited in both range of agents which they can carry and target accessibility, this is even more so for other cells (for example leukocytes, hepatocytes) which function as vehicles of agents they contain naturally (Table 1). Nonetheless, Brady's suggestion⁸⁹ of transporting enzymes past the blood-brain barrier in leukocytes (known to percolate through the cerebral tissue after emigrating from post-capillary venules) may render such cells valuable therapeutic tools.

How realistic then is the concept of an effective multi-purpose carrier? For reasons outlined earlier, it is apparent that there is indeed a requirement for a three-dimensional system capable of containing agents within a well-protected space and amenable to modifications, both structural and functional. In retrospect, it seems unlikely that any other natural materials would have been as appropriate building blocks for the carrier as insoluble polar phospholipids.

These, when confronted with water, form highly ordered assemblages of one or several concentric lipid bilayers known as liposomes^{80,91} and which by the use of simple techniques⁹² can accommodate a wide variety of water soluble substances (Table 1). Alternatively, lipid soluble materials including the hydrophobic regions of macromolecules such as proteins, can be inserted into the lipid space in the bilayers⁹³. Several of the physical properties of liposomes can be varied at will: size (radius) can be adjusted from about 12 nm for monolamellar liposomes to up to several microns for the multilamellar version, and a negative or positive surface charge can be imposed by the incorporation of charged amphiphiles⁹². Control of permeability to entrapped substances, and of stability is also feasible by the addition of a sterol or other lipids into the liposomal structure⁹². Finally, manipulation³⁵ of the surface characteristics of liposomes (see below) will enable them to recognise specific cells.

Following the demonstration^{94,97} that injected liposomes control both the rate of elimination from the circulation and tissue distribution of entrapped agents, a considerable amount of work has been carried out in regard to the possible role of liposomes as carriers in biology and medicine³⁵. For example, intravenously given liposomes by and large remain intact in the blood and entrapped non-diffusible agents (for example enzymes) are transported predominantly into the liver and spleen^{97,98}. Entrapped small molecules such as 5-fluorouracil⁶² and penicillin⁷⁰ diffuse from the carrier into the blood while others (for example actinomycin D⁷⁰, colchicine⁶⁹) are retained and follow the carrier to its destination. We have found that retention of daunomycin and melphalan by liposomes is greatly augmented when the two drugs are bound to DNA and polyglutamic acid respectively (G. Gregoriadis, S. C. Scott and P. N. Davisson, unpublished work). Rates of liposome clearance from the site of injection as well as penetration of target areas can easily be varied by controlling liposomal

size, surface charge and lipid composition. Thus, intravenously injected small liposomes circulate in the blood for longer periods⁶⁹ and, because of their size, can reach malignant tissues⁷⁰. Similar liposomes given intramuscularly enter the lymphatic nodes draining the injected tissue⁷¹. On the other hand, liposomes composed of phospholipids resistant to gut phospholipases at the body temperature are capable of transporting insulin from the gut into the blood circulation and of reducing blood glucose levels in normal and diabetic rats⁷². Indeed a considerable body of experimental evidence, discussed in detail elsewhere⁷³, has now made possible a realistic appraisal of the carrier potential of liposomes in the treatment of diseases as diverse as cancer, diabetes and lysosomal storage diseases and also in preventive medicine (for example as immunological adjuvants).

Although manipulation of the physical parameters of liposomes can modify to some extent the pattern of their tissue distribution⁷⁴, a method for targetting liposomes would vastly expand their potential. A few attempts for

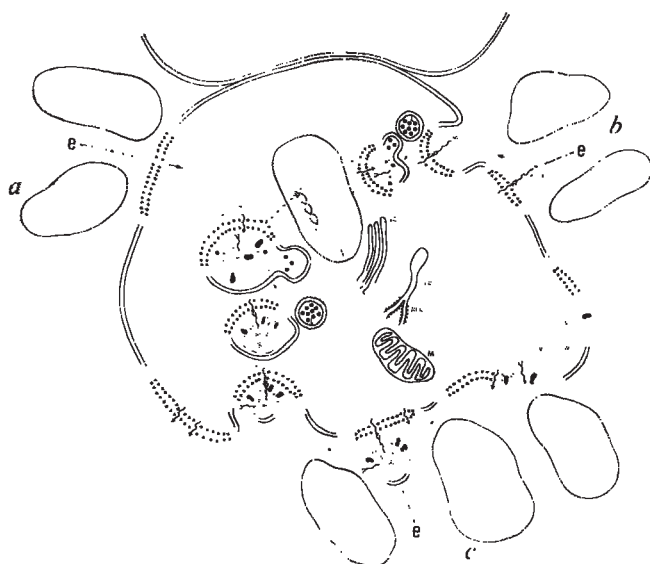


Fig. 1 Interaction of carrier-associated drugs with cells. A drug molecule (*) presented to a cell in its free form can enter the interior non-specifically by mechanisms such as diffusion and endocytosis⁷⁵ (a). Untoward reactions may occur because of the availability of the free drug to, and its interaction with, the environment (e). In b, interaction of the carrier-drug complex (α-α) with a carrier-recognising receptor (→) on the cell membrane induces endocytosis of the complex and its subsequent interiorisation into a lysosome where lysosomal esterases (●) liberate the drug. Other mechanisms of action of the carrier-bound drug are also possible⁷⁶. Carrier-bound drug molecules are again available to, and can interact with, the environment but owing to the size of the complex and its affinity for the target, diffusion to non-target areas is reduced. c, A drug-containing monolamellar liposome recognises the target cell through a homing macromolecule (—<) inserted on the liposomal surface and which binds to the relevant cellular receptor (→). Contact with the cell is followed by endocytosis of the carrier (arrow pointing to the left) and its subsequent localisation in the lysosomes. Lysosomal esterases (phospholipases?) disrupt the bilayer and free the drug which can either act locally or, after escape from lysosomes, reach other cell compartments and interact with molecular targets (for example interaction of actinomycin D with DNA in the nucleus). Alternatively the liposome, upon fusion with the cell membrane (arrow pointing to the right), introduces its water-soluble contents into the cytoplasm and makes them available to the remainder of the cell. Lipid-soluble agents (●) carried by the lipid bilayer of the liposome are transferred after fusion into the framework of the cell membrane. Liposome associated drugs, being surrounded by the lamellae, are not available to the environment. N, nucleus; GA, Golgi apparatus; SER and RER, smooth and rough endoplasmic reticulum; M, mitochondrion; L, lysosome.

improving target selectivity have already been made: after *in vitro* exposure of phagocytes from *Mustelus canis* and a Tay Sachs disease patient to liposomes coated with aggregated isologous immunoglobulin, there was considerable improvement in the cellular uptake of the liposome-associated horseradish peroxidase⁷⁷ and hexosaminidase A (ref. 43) respectively. It was assumed that association of liposomes and their enzyme contents with phagocytes was mediated through the Fc portion of the extraliposomal immunoglobulins. Other workers⁷⁸ found that incorporation into the liposome structure of a sialoglycoprotein extracted from the erythrocyte membrane, could facilitate *in vitro* binding of such liposomes to erythrocytes in the presence of lectins. Our studies have shown that homing of liposomes is also possible *in vivo*. Thus, desialylated fetuin (which, as mentioned earlier, exhibits affinity for the hepatic parenchymal cells) incorporated on to the liposomal surface was found⁷⁹ to mediate uptake of the liposome-associated bleomycin by the relevant receptors on the liver cell surface.

The possibility of a more general method for the selective uptake of liposomes by cells is under investigation in my laboratory. Our approach⁸⁰ is based on the anchoring of native anti-target IgG, immunoglobulins, through their Fc portions, into the liposomal lipid phase. The Fab moiety seems to extend extraliposomally (G. Gregoriadis and E. D. Neerunjun, unpublished work), and is capable of recognising, and associating with, the respective antigens on the target cell surface. Before trying to raise antibodies to cell specific antigens we experimented with the possibility of using whole cells for antibody production. It was hoped that the presence of cell-specific strong antigenic determinants on the cell surface would produce antisera rich in antibodies to such antigens and therefore reasonably specific for the parent cells. Indeed, after exposure of HeLa cells and human skin fibroblasts to liposomes containing ¹¹¹In-labelled bleomycin and bearing on their surface ¹²⁵I-labelled IgG raised against whole HeLa cells, the uptake of both radioactivities by HeLa cells was 25 times greater than that with fibroblasts⁸⁰. On the other hand, after exposure of fibroblasts to the same liposomes now bearing anti-fibroblast-labelled IgG, uptake of the two radioactivities was five-fold greater than with HeLa cells⁸⁰. As both cell types exhibited similar rates of uptake of bleomycin-containing liposomes coated with nonspecific IgG, it was concluded that cell-specific IgG on the liposomal surface was mediating selective uptake of the carrier and its drug contents. In practical terms, this approach has not only made the need for production of IgG against purified cell-specific antigen doubtful, it also suggests that purification of anti-target IgG from the multitude of other IgG molecules in the serum may be unnecessary; assuming a random distribution of the IgG molecules within the liposomal population, each individual liposome would be expected to possess on its surface some of the target-specific IgG. These specific molecules would then mediate the interaction of the associated carrier and the target. With regard to allergic reactions to the IgG used for homing, it is conceivable that insertion of the Fc portion of the immunoglobulin molecule into the lipid bilayer (which still allows the Fab portion to recognise its antigen) could mask this strongly antigenic site of the protein itself and thus prevent its interaction in the circulation with the respective anti-IgG (Fc) antibodies. Such avoidance of antigen-antibody interaction has been already observed for liposome-associated diphtheria toxoid⁸¹, asparaginase⁸² and insulin⁸³.

Effective direction of liposomes to target areas in the body will depend crucially on the circumvention of at least two obstacles. First, and when the target is not the hepatic parenchymal cells or in the confines of the reticuloendothelial system, for which conventional liposomes show preference, it will be necessary to reduce hepatic and splenic interference. Modest attempts^{84,85} have shown that

this is possible. Second, the size and composition of liposomes must be manipulated in a way that will allow passage of the carrier through a variety of anatomical barriers. This too is under investigation⁴⁸ but as there is a minimum (12 nm) in the radius of liposomes, there will be limitations. Other mechanisms, however (for example the use of leukocytes⁴⁹), by which liposomal contents can be somehow transported into restricted areas cannot be excluded.

Provided that such difficulties can be overcome, it would be possible for specialised liposomes to seek and reach their cellular target site, become associated with it and deliver their contents. Two mechanisms of cell-liposome association are currently recognised³⁵, namely endocytosis¹⁰⁰⁻¹⁰² and fusion^{45,73,103-105} (Fig. 1c). Direct as well as circumstantial evidence³⁵ suggests that the former mechanism occurs both *in vitro*^{106,38} and *in vivo*^{101,102,107} with a variety of actively or not so actively endocytosing cells. Because of this process, liposome-associated agents end up in the lysosomes where they are liberated and assuming such agents retain their activity in the lysosomal milieu, they can then act locally^{108,38,108}. Alternatively, and when the physical properties of the agents allow it, these can escape by diffusion and reach other cell regions⁸¹. There is now considerable evidence^{73,103-105,109} to suggest that under certain conditions *in vitro* the liposomal bilayers can, in addition to being endocytosed, fuse with the cell membrane (Fig. 1c). This can either mediate incorporation of lipid soluble substances (located within the lipid phase of liposomes) into the cell membrane¹¹⁰ or introduction of water soluble substances (entrapped in the aqueous space) in the cytoplasm⁷³.

Selective association of liposomes with cells could bring agents with their own selectivity for action near intracellular molecular targets. For instance actinomycin D or daunomycin originating from lysosomes (endocytosis) or from the cytoplasm (fusion), could enter the nucleus, bind to DNA and inhibit DNA directed RNA synthesis⁹¹. Similarly, agents in the vicinity of mitochondria or other organelles could, because of a specific affinity, bind to respective components and exert their effect. It thus appears that targetting of drugs with liposomes could proceed in two steps. In the first, the carrier would recognise the general area of action and subsequently enter it. Following their liberation from liposomes, drugs could then proceed for a finer target recognition at the molecular level (Fig. 1).

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articles

Additional results on palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya

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The magnetostratigraphy of the hominid-bearing sediments exposed east of Lake Turkana has been strengthened by new palaeomagnetic results. Ages obtained from several tuffs by the $^{40}\text{Ar}/^{39}\text{Ar}$ method suggest an approximate match between the observed magnetozones and the geomagnetic polarity time scale; however, the palaeomagnetic results are also compatible with a younger chronology suggested by conventional K–Ar dating of the KBS Tuff.

BROCK and Isaac¹ have reported palaeomagnetic polarity determinations from the Koobi Fora Formation, exposed on the north-east side of Lake Turkana (formerly named Lake Rudolf), Kenya (Fig. 1). Because these Pliocene and Pleistocene deposits have yielded rich assemblages of fossil vertebrates, including the remains of very early humans, the ages of these beds have received considerable attention^{2–4}. The Koobi Fora beds have also yielded a large collection of stone artefacts representing at least two tool-making industries^{5,6}. The palaeomagnetic study complemented a framework of radiometric ages determined from five tuffs interbedded within the Koobi Fora formation⁷. After Brock and Isaac's results were published, one of the tuffs, the KBS, was redated in another laboratory using the conventional K–Ar method, and a younger age was reported⁸. We have now obtained additional palaeomagnetic results which warrant a reevaluation of the magnetic stratigraphy.

The palaeomagnetic study of Brock and Isaac included 247 oriented specimens collected from four stratigraphic sections within the Koobi Fora Formation. The sedimentary deposits consist of fluvial sandstones and siltstones interbedded with lacustrine mudstones that formed near the eastern shore of ancient Lake Turkana. The palaeomagnetic results were tied to local, discontinuous outcrops of tuffs,

each of which has been tentatively correlated with one of five ash beds that are believed to be widespread marker units within the Koobi Fora area. Listed from oldest to youngest, these tuff horizons have been named: Suregei, Tulu Bor, KBS, Koobi Fora and Chari. The proposed correlation of tuffs should be considered as a working hypothesis subject to revision. Further work is now in progress and it is evident that some amendments to the published correlations^{9–12} are called for (I. Findlater, J. M. Harris, T. White and R. E. F. Leakey, personal communication.).

A consistent sequence of polarities was established from the four stratigraphic columns, and using the previously published age of 2.6 ± 0.26 Myr for the KBS Tuff¹³ as a fixed point, this sequence was matched with parts of the geomagnetic polarity time scale. In this interpretation part of the lower member of the Koobi Fora Formation, bracketed by the KBS and Tulu Bor Tuffs, was assigned an age of 2.6–3.2 Myr, while the upper member was assigned an age between 1.2 and 1.8 Myr. As reversed rocks were not found between the Koobi Fora Tuff and the KBS Tuff, the interpretation required that sediment representing 600,000 yr of the early Matuyama epoch had been removed from the section, possibly by uplift and erosion after deposition of the KBS Tuff. The subsequent publication⁷ of ages for the other tuffs, namely, Tulu Bor (3.18 ± 0.09 Myr), Koobi Fora (1.57 Myr) and Chari (1.22 ± 0.01 Myr) provided strong support for the palaeomagnetic interpretation.

The basis of the initial polarity interpretation was the KBS Tuff, the age of which is now disputed. An age of 2.6 ± 0.26 Myr was initially assigned to the KBS Tuff on the basis of apparent age spectra determined by the $^{40}\text{Ar}/^{39}\text{Ar}$ method, which entails step-wise heating of specimens. It was thought that the conventional K–Ar method could not be applied successfully to the Koobi Fora tuffs because the ash had lost some argon, due possibly to widespread hydrothermal events¹⁴. Conventional K–Ar

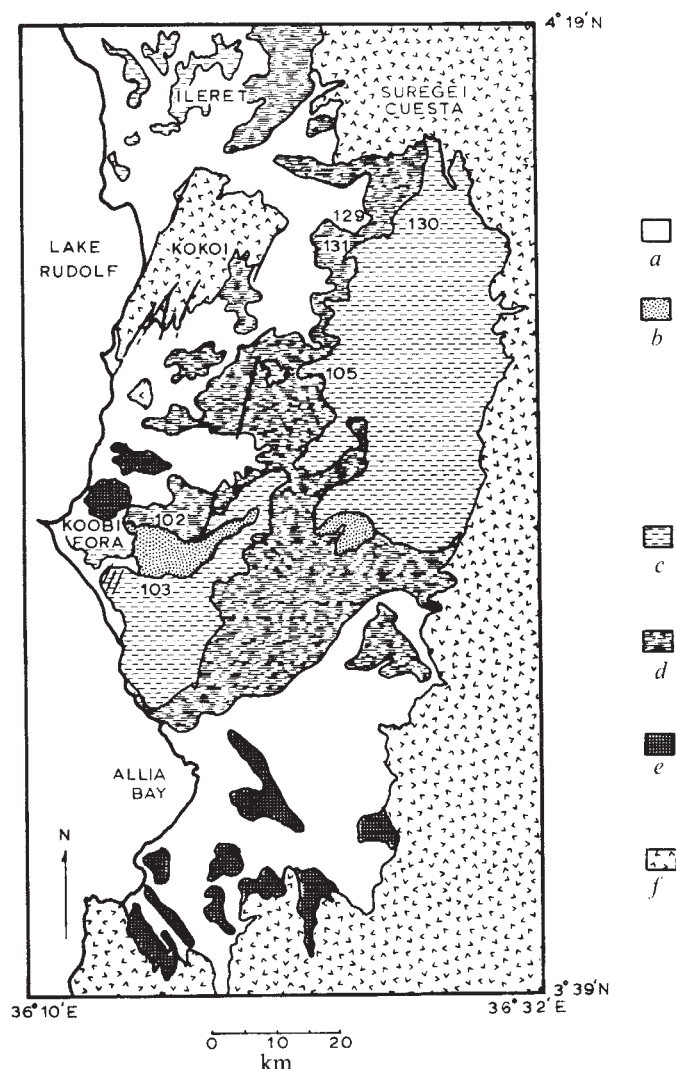


Fig. 1 Geological map of the Koobi Fora region in northern Kenya, showing palaeomagnetic sampling area (after Vondra and Bowen (ref. 11)). *a*, Alluvium, beach sand; *b*, Galana Boi beds. *c-d* Koobi Fora Formation. *c*, Upper member; *d*, lower member; *e*, Koobi Fora Formation; *f*, volcanic rocks undifferentiated.

dating has now yielded significantly younger ages⁸. Glass and sanidine separates taken from the same sample have remarkably similar ages; nine specimens from areas 105 and 10 gave an average age of 1.60 ± 0.05 Myr and six specimens from area 131 gave an average age of 1.82 ± 0.04 Myr. These results combined with trace element analysis indicated that the tuffs exposed in areas 105 and 131 might be from two eruptions separated in time by 200,000 yr. The high precision and concordancy of these dates determined from two materials with different crystal structures provide strong evidence that the tuff has not lost radiogenic argon. Additional $^{40}\text{Ar}/^{39}\text{Ar}$ measurements¹⁵ and fission track age determinations¹⁶, however, provide evidence in favour of the earlier date for the KBS Tuff. Thus the chronology of the Koobi Fora Formation remains open to discussion, and alternate interpretations of the magnetostratigraphy, enhanced by 800 additional samples, are presented here.

Additional palaeomagnetic results

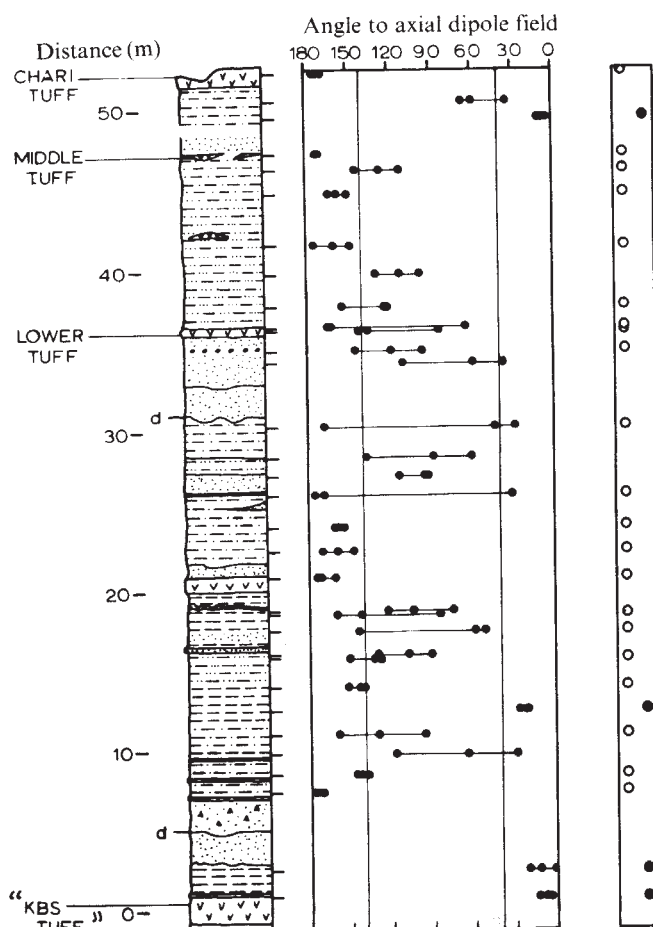
We have determined magnetic polarities of six new stratigraphic sections, spanning the following stratigraphic

intervals (Figs 1 and 4): (1) area 131: 'Tulu Bor'-KBS Tuff; (2) area 105 and 116: 'Tulu Bor Tuff'-KBS Tuff (type section); (3) Ileret: 'KBS Tuff'-Chari Tuff (type section); (4) area 129 and 15: Suregei Tuff-Tulu Bor Tuff (type section); (5) area 102: 'Suregei Tuff'-Koobi Fora Tuff; (6) area 103: 30-m column just below the Koobi Fora Tuff (type section).

Because the identities of tuffs in localities other than the type areas are hypothetical, the above list indicates whether or not the palaeomagnetic column is attached to a type section. In the remainder of this paper, tuffs which have not been traced back to the type section with certainty are flagged with quotation marks.

The sampling technique has been described by Brock and Isaac¹. The magnetic polarity of each horizon was determined from three oriented cubes ($2\text{ cm} \times 2\text{ cm} \times 1.5\text{ cm}$) with a 5 c.p.s. flux-gate spinner magnetometer. One sample from each horizon was progressively demagnetised with an alternating-field (AF) up to 300 Oe, then the optimum field was selected as that which minimised the change in magnetic direction between successive demagnetisation steps. The remaining samples were demagnetised at the optimum field determined from the pilot sample. Typical optimum fields of these samples ranged between 100 and 200 Oe, with the remaining magnetisation averaging 10–25% of the initial natural magnetisation. Almost all of the specimens had acquired

Fig. 2 Palaeomagnetic results from the Ileret section. The angular difference between the cleaned palaeomagnetic direction and the normal axial dipole field is plotted for each sample. On the right the polarity of each horizon has been interpreted as normal (dot) reversed (open circle), or indeterminate (no symbol) from analysis of three samples. Disconformities are indicated by *d*. The tuff correlated as the 'KBS' is the uppermost of a pair of tuffs separated by several metres of sediment. This same upper tuff was dated by Curtis *et al.*⁸ at 1.60 ± 0.05 Myr.



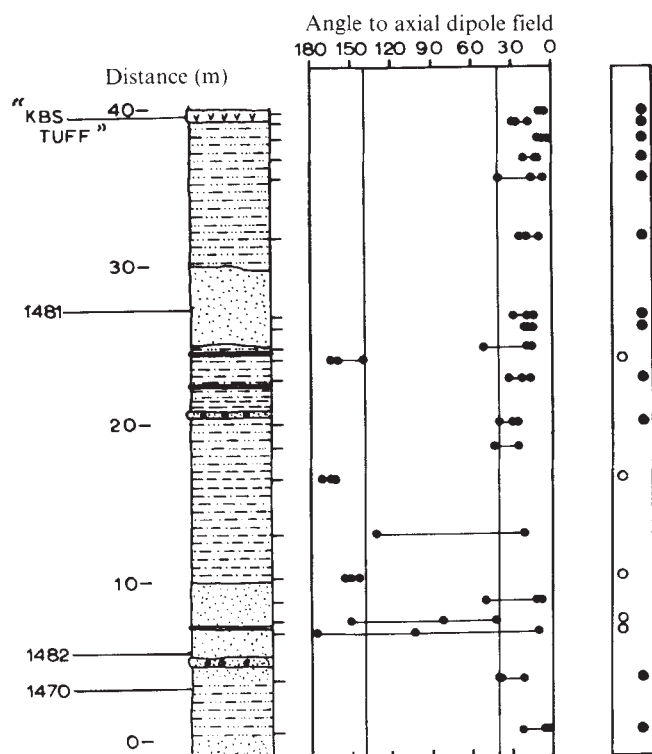


Fig. 3 Palaeomagnetic results from the lower member of the Koobi Fora Formation in area 131. Stratigraphic positions of hominid sites ER-1470, ER-1482, and ER-1481 are indicated³. The Tulu Bor Tuff is believed to be located approximately 6 m below the base of this section. The tuff correlated as the 'KBS Tuff' in this column was dated by Curtis *et al.*⁸ at 1.82 ± 0.04 Myr. The possibility cannot be eliminated that this tuff is the lateral equivalent of a minor tuff that occurs below the type KBS Tuff in area 105.

viscous magnetisation parallel to the normal axial dipole field, and this component was effectively removed by AF demagnetisation of 50–100 Oe.

In the treatment of the preliminary columns, Brock and Isaac¹ defined normal and reversed magnetic directions as those which deviated less than 50° from the axial dipole ($D=0^\circ$, 180° ; $I=\pm 8^\circ$). Directions lying outside these 50° cones were regarded as intermediate. In a few cases the cleaning procedure produced erratic changes of direction between successive steps, so these samples were classified as giving no clear result. In order to give a better representation of the range of dispersion within horizons we have modified this analysis by computing for each sample the angle between the cleaned palaeomagnetic direction and the normal axial dipole field ($D=0^\circ$, $I=8^\circ$). Because all of the sampling localities are located within a 30-km radius, magnetic directions rather than virtual geomagnetic poles (VGP) were analysed. As examples, the palaeomagnetic results from columns in areas 131 and Ileret are shown in Figs 2 and 3.

We have defined the polarity of each specimen using cones of confidence centred about the normal and reversed axial dipole directions. Given a period of 10^4 years or more, the time-averaged direction of the Earth's field at a given site is very close to the axial dipole direction, although at a given instant changes in the non-dipole field may displace the field vector away from the axial direction. At the latitude of Koobi Fora (4°N) the angular standard deviation is 18° , based on a model which assumes non-dipole dispersion similar to that of the present field¹⁷. Palaeomagnetic evidence from Tertiary lavas indicates that this value of the standard deviation is in accord with ancient field behaviour^{18,19}. We have used an angle (40°) slightly larger than two standard deviations as the radius of the

95% confidence cone centred on the axial dipole in order to define the polarities as follows: (1) If the angle to the axial dipole lies between 0° and 40° the specimen has normal polarity. (2) Between 140° and 180° , the specimen has reversed polarity. (3) Between 40° and 140° the specimen has intermediate polarity.

Even after AF cleaning, some horizons gave three widely scattered directions, and in extreme cases the horizons contained normal and reversed polarities. This inconsistency indicated that some samples had acquired secondary remanence which was not completely removed by AF demagnetisation. Therefore the polarity of each horizon was interpreted from three specimens according to the following rules: (1) If one or more specimens had reversed directions, the horizon was assigned reversed polarity. (2) If all three specimens had normal directions, the horizon was assigned normal polarity. (3) If a horizon had one or more intermediate specimens and no reversed specimens, the results were rejected and the horizon's polarity was said to be indeterminate. Intermediate directions may record true palaeofield directions during polarity transitions or excursions, or they may be due to the superposition of primary and secondary magnetic components with opposite polarities. Because of this ambiguity, intermediate directions were ignored in the interpretation of the magnetic stratigraphy.

This scheme deliberately favours reversed directions. Our reason for adopting it is that many Koobi Fora specimens have acquired an overprint of stable secondary remanence with normal polarity. This normally polarised secondary remanence may have been acquired by two processes; it may have originated as viscous remanence due to recent exposure in the Earth's field or it may have originated as chemical remanence by oxidation of magnetite during weathering. Because the Earth's field has been normally polarised essentially all of the time since 700,000 BP we would expect the viscous magnetisation to be normal. Recent weathering of outcrops may cause chemical changes that also impart normally polarised chemical remanence. For these reasons, reversed magnetisation is less likely to represent overprinting due to viscous magnetisation or weathering and therefore is more reliable for purposes of palaeomagnetic stratigraphy. Some specimens may have acquired post-depositional chemical remanence during periods of reversed polarity; however, at Koobi Fora long intervals of normally polarised rocks contain only a few intermediate and reversed specimens; they tend to be entirely normal. On the contrary, horizons with widely scattered directions are confined almost entirely to beds that have reversed polarity. This indicates that for the most part secondary remanence is normally polarised. In short, reversed magnetisation indicates that the rocks formed when the field was reversed whereas the converse is not always true for normal magnetisation. Good examples of the relations defined above can be seen in the Ileret and area 131 sections (Figs 2 and 3). It was for the above reasons that reversed directions were given more weight than normal directions in the interpretation of the magnetic stratigraphy.

Figure 4 summarises the magnetic stratigraphy of the Koobi Fora Formation, including the preliminary results¹ in columns marked with arrows. Two incomplete sections, Ileret 6A and area 103, which were presented by Brock and Isaac, have been superseded. Although some inconsistencies are apparent between the sections, a coherent pattern may be seen which provides a basis for making correlations with the geomagnetic polarity time scale. Besides extending the magnetostratigraphy down to the Suregei Tuff, the new results have led to some revisions of the preliminary magnetostratigraphy. For example, in area 105 between the 'Tulu Bor' and the type KBS Tuffs, Brock and Isaac found evidence for two reversed zones separated by a short

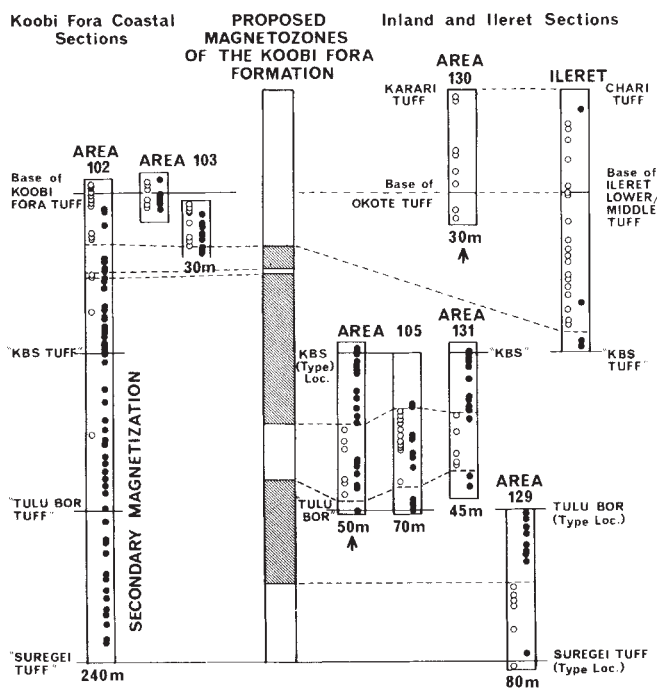


Fig. 4 Summary of palaeomagnetic stratigraphy of the Koobi Fora Formation. The stratigraphic sections are correlated by several key tuffs, placed on an arbitrarily spaced grid. The actual thicknesses are shown at the bottom of each stratigraphic section. Because the correlations of tuffs outside the type localities are provisional these correlations are flagged with quotation marks. Normal polarities (dot) and reversed polarities (open circle) define magnetozones shown in the generalised column. Columns previously reported by Brock and Isaac¹ are marked with an arrow.

normal zone. Resampling of the same interval in areas 105 and 131 showed the presence of sporadic normal horizons within the thick reversed zone. In keeping with our assumption that reversed magnetic directions are more reliable than normal ones, we have disregarded those isolated normal horizons in constructing the generalised magnetic column in Fig. 4. Therefore, we now regard the reversed horizons below the KBS Tuff as part of one magnetozones.

The inconsistencies serve as a warning that palaeomagnetic dating, like K-Ar dating, is subject to errors due to geochemical processes. For example, the lower portion of the Area 102 column (Fig. 4) is normally polarised, whereas sections of overlapping age in areas 129, 131 and 105 have several bands of reversed polarity. In area 102 the oldest sediments consist of brittle, grey claystones deposited in deep water within a rapidly subsiding graben. In contrast the equivalent deposits exposed in areas 105 and 131 are composed of fluvial and shallow lacustrine sediments. Apparently, the original palaeomagnetic signal carried by the area 102 clays was erased, perhaps by chemical alteration of the detrital magnetic minerals during burial. In recent time the clays may have acquired stable chemical magnetisation of normal polarity. Palaeomagnetic results from the lower portion of the area 102 column were therefore largely discounted. As a general rule, the least reliable palaeomagnetic columns of the Koobi Fora formation were located in hard lacustrine clays exposed near the present shoreline. The most consistent results were obtained from deltaic and shallow lacustrine sediments exposed near the eastern margin of the Koobi Fora beds.

In all columns, however, mixed polarities and scattered magnetic directions were most apparent in poorly consolidated sandy beds where the mean grain size exceeded 50 μm . For example, the reversed magnetozones from area 103 is interspersed with isolated normal horizons and many

of the reversed horizons include one or two specimens with normal or intermediate polarity. Most of these unreliable results were determined from friable, coarse-grained sediments. In the area 102 column, scattered magnetic directions occur between the 'KBS' and 'Tulu Bor Tuffs'. This part of the column largely consists of sandstone. It is possible that the entire column from area 102 is affected by remagnetisation, and that in Fig. 4 we have overestimated the true thickness of the normal magnetozones which lies above and includes the 'KBS'.

Post-depositional normal polarity may be present in other areas, so the magnetic stratigraphy should be interpreted cautiously, especially where short normal magnetozones are present. We have attempted to determine which magnetozones correspond to real changes in geomagnetic polarity by repetitive sampling of palaeomagnetic columns throughout the Koobi Fora Formation.

There is an element of ambiguity in palaeomagnetic dating because it involves recognition of a pattern composed of only two elements; the identify of a particular time interval is determined from the relative thicknesses of adjacent polarity zones. Where deposition has been steady and where the magnetic recording process has been of good fidelity, palaeomagnetic ages can be assigned with the known reversal time scale. Hiatuses and changes in the deposition rate, however, which are common in the fluvial and lacustrine facies of the Koobi Fora Formation, have distorted the polarity sequence. Additional age controls provided by the fossil record and by K-Ar dating are needed to provide an approximate match with the known reversal time scale. The palaeomagnetism can then be used to define the ages of some of the key strata.

Interpretation of magnetic stratigraphy

We have constructed a generalised stratigraphic column which shows the relative positions of the tuffs and the observed magnetozones of the Koobi Fora Formation (Fig. 5). With control provided by the framework of radiometric ages of the tuffs, the magnetozones are correlated with the geomagnetic polarity timescale²⁰⁻²². As the age of the KBS Tuff remains under discussion we propose two possible interpretations of the Koobi Fora magnetic stratigraphy. Interpretation 1, proposed originally by Brock and Isaac¹, is based on a KBS age of 2.6 Myr obtained by the ⁴⁰Ar/³⁹Ar step-heating method. Interpretation 2 is an alternative which is in accord with the KBS complex age of 1.60-1.82 Myr obtained by Curtis and others⁸ who used the conventional K-Ar technique.

In both interpretations the age of the upper member, which lies above the KBS Tuff, is between 1.2 and 1.8 Myr; however, the age of the top boundary of the lower member differs by 1 Myr. If interpretation 1 is accepted, the first reversed magnetozones below the KBS Tuff correlates best with the combined Mammoth and Kaena events, 2.8-3.1 Myr. Accordingly, the reversed interval below the Tulu Bor Tuff correlates with the end of the Gilbert epoch, 3.3-3.7 Myr. Because a substantial interval of reversed polarity is missing between the KBS Tuff horizon and the 1.61 Myr polarity boundary, this interpretation requires that sediments of early Matuyama age, 1.8-2.4 Myr, are not present in the Koobi Fora area. It has been proposed by Brock and Isaac that this time gap is due to uplift and subsequent erosion. Indeed, a disconformity was mapped in area 105, area 103, and Ileret just above the 'KBS Tuff'⁵. Whether the corresponding hiatus is as long as the required 600,000 years is uncertain, however, and this uncertainty remains a major weakness of the chronology.

Interpretation 2, which is consistent with an age of 1.6-1.8 Myr for the KBS Tuff complex, assigns an age of 1.8-2.4 Myr to the first reversed magnetozones below the KBS Tuff. Reversed horizons below the Tulu Bor Tuff are in accord with the combined Mammoth and Kaena events

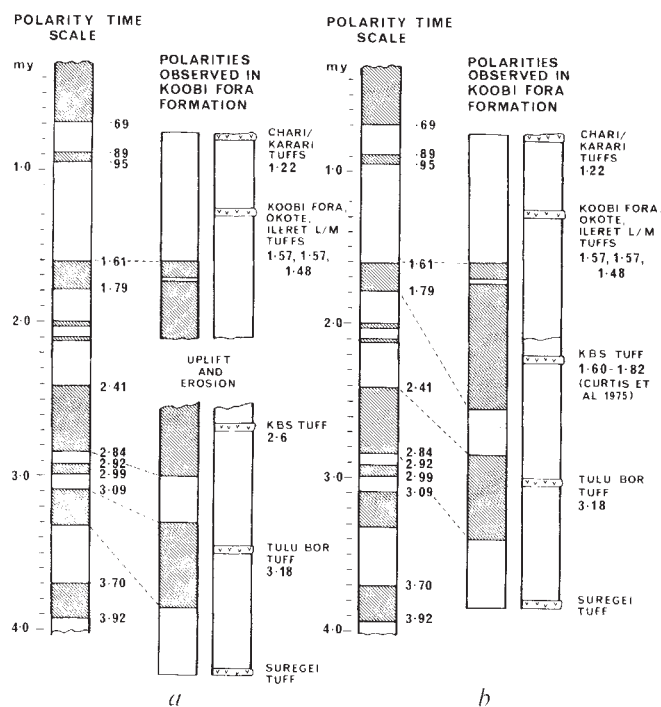


Fig. 5 Two possible correlations of the magnetozones observed in the Koobi Fora Formation with the geomagnetic polarity time scale. *a*, Interpretation 1 is based on $^{40}\text{Ar}/^{39}\text{Ar}$ dating of the KBS Tuff (2.6 Myr) by Fitch and others². *b*, Interpretation 2 is based on conventional K-Ar dating of the KBS Tuff, which gave two ages, 1.60 Myr in area 105 and 1.82 Myr in area 131⁸. All other ages were determined by the $^{40}\text{Ar}/^{39}\text{Ar}$ method.

(2.8–3.1 Myr). In contrast with the original chronology, interpretation 2 reduces the age of the reversed magnetozones between the Tulu Bor and KBS Tuff by 0.7–1.0 Myr and increases its duration from 0.3 to 0.6 Myr. Interpretation 2 also suggests that the Tulu Bor Tuff is younger than 2.8 Myr, contradicting the tuff's radiometric age of 3.18 ± 0.09 Myr, and this must be accepted as a major weakness of the interpretation.

Fossil mammal assemblages offer another means of calibrating the polarity sequence at Koobi Fora against other well-dated localities in East Africa. Palaeontologic, palaeomagnetic, and radiometric ages have been obtained from the Pliocene and Pleistocene Shungura Formation, located along the Omo River in southern Ethiopia²³. The occurrence of the fossil pig *Mesochoreus limnetes* in both the

Koobi Fora and Shungura Formations helps in the correlation of the magnetozones between the two areas. Cooke and Maglio²⁴ reported that *Mesochoreus limnetes* from below the KBS Tuff at East Turkana correlates best with Shungura Member F which has a K-Ar age of approximately 2.0 Myr (ref. 23). The biostratigraphic range of a single species can vary between regions depending on palaeoecological conditions, so the above correlation must be weighed against other lines of evidence. Recent collecting in these areas has provided a larger sample of the fossil fauna which is now being analysed (J. M. Harris, personal communication). Note that neither geophysical methods nor biostratigraphy yet provide an unequivocal chronology for the Koobi Fora Formation.

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Note added in proof: Continuing studies show partial error in tuff correlations (Fig. 4): (1) Identities of tuffs are unknown in the lower portion of area 102 column, so we can no longer assume that this section was overprinted with normal polarity. (2) A stratigraphic gap could exist between the bottom of column 105 and the top of column 129 because area 105 basal tuff cannot be firmly correlated with the type Tulu Bor Tuff of area 129. 'Area 129' column is a composite: the upper portion was from area 129, the lower, from the adjoining area 15.

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Aggregation, migration and population mechanics

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A concept is developed for the regulation of populations by density-dependent movement, rather than by overt competition alone. Fitness is seen as maximising the reproductive advantage of a balance between migratory and congregatory behaviours. Population density is shown to be spatially, as well as temporally dynamic and a mechanism is proposed that accounts for observed spatial behaviour.

THE Malthusian concept of population is contained in the basic proposition that, with full census information, population change can be assigned to either reproduction or mortality¹. This proposition is consequently the basis of Darwinian fitness measured in terms of reproduction and survival², and of population models for competition^{3,4} and predation^{5,6} that are primarily concerned with equilibrium conditions^{7,8}. Populations are seen as persisting through time owing to the check on

multiplication imposed by such familiar Malthusian⁹ restraints as disease, predation and resource limitation. Key factors^{10,11} direct our attention to the causes and time of death, but not to the place of death nor to its distance and direction from the place of birth. Lacking this component of movement in real space, it seems to us that populations are static¹², not dynamic. They can neither function nor evolve unless they include the controlled mobility that enables organisms to select a place to live and so survive and reproduce. This mobility we shall equate largely, but not entirely, with internal or external migration using the word in a wider sense than that familiar in ornithology.

Migration and static population concepts

Until recently migration has often been regarded as an idiosyncrasy to be included with unaccountable mortality in life tables based on census information from a restricted area. Yet migration is not synonymous with mortality. Re-examination of old census records now reveals more extensive, successful, internal migration in man than was formerly appreciated^{13,14}. Among insects, many species formerly regarded as sedentary have been found to be vagile¹⁵. For example, *Drosophila* seem non-migratory as compared with the monarch butterfly¹⁶, and their flight behaviour is well adapted to minimising dissemination^{17,18}. Even so, their migratoriness is now known to be considerably underestimated in the Dobzhansky-Wright models that so influenced concepts of population mobility in genetics^{19,20}. New evidence from bird ringing shows long distance movements in territorial species formerly considered extremely sedentary, the European blackbird, for example²¹, and, since young birds rarely occupy their parents' nests, they must migrate to establish a new territory. Dispersal in small mammals has been related to population cycles²² and the zoo syndrome has been called "frustrated dispersal"²³ in species not commonly thought of as migratory.

Segregation of migration from dispersal obscures their common population function. In birds, fish and especially in insects, whose complex life-histories enable the various aspects of migration to be analysed in most detail^{24,25}, much is now known about ontogenetic²⁶, physiological²⁶ and behavioural²⁷ processes that initiate movement and about its evolutionary origins²⁸ and ecological function²⁹, as well as about the more striking, but in this context less relevant, orientation^{30,31} and navigation³² behaviour. Gene migration models³³⁻³⁵, like population models³⁶, have not yet made due allowance for the sophisticated migration control mechanisms in which the experience of parents and grand-parents³⁷, as well as population density³⁸, affect the migrant status of the individual. These are most clearly evident in the labile³⁹ migratory polymorphisms, remote from genetic heterogeneity, in clonal populations of aphids⁴⁰. As a result, little light has been shed on the role of migration in the dynamics, and hence in the fundamental concept, of population.

Concepts of population are not consistent¹¹. A lack of rigour in distinguishing total population from population density can imply uniform distribution of density in space, whereas the actual spatial distribution of few organisms is known¹². Again, key factors differ for the same species in different places and affect different stages in the life history. This makes models for the whole species population incomprehensible. If, however, a model is considered to represent a separate element of the population, equivalent to the mean density at a certain place, then growth rate can be equated with unity, as required by basic theory^{9,13}, only if there is no population flow between such places.

But we doubt that populations are often like that. All populations are spatially fluid in some measure because movement is a fundamental biological response to adversity. Nor is population density distributed uniformly in space. A valid concept of population must encompass both the more settled species, like the red grouse say⁴¹, to which the current

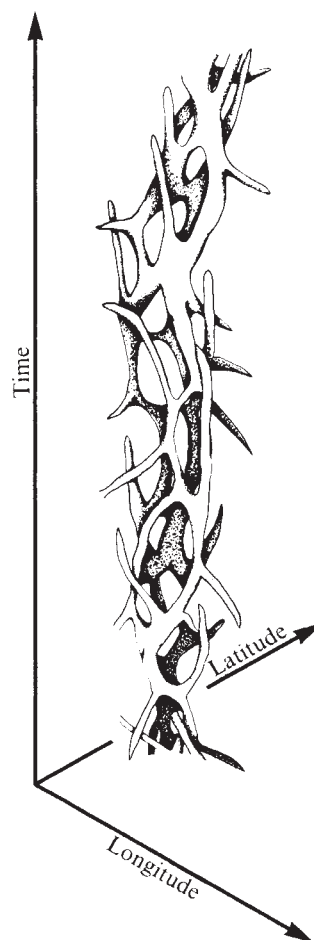
models appear to be most suited, and the highly volatile species, like aphids, for which local stability in time seems almost irrelevant because of the persistent change of place.

A spatial concept for population and some evidence

The classical model for the whole population of a species changing only through time was the phylogenetic tree. If we treat the anatomy of a real population as being in three dimensions, latitude longitude through time, we visualise its internal structure to be reticulate, not unlike the fern stele in Fig. 1 in which density changes constantly through time and space. So, spatial sections through this anatomical model at successive time intervals differ slightly, or markedly, depending upon the time scale. (This has much in common with MacArthur's¹⁵ r - K continuum, and with Southwood's¹⁶ τ/H ratio.)

After monitoring the changing distribution of density of hundreds of species of Lepidoptera throughout Great Britain for almost a decade⁴², we can show that growth cycles in different local population elements are normally asynchronous, as in Ford's marsh fritillaries⁴³, combining with movement to change the pattern of density in space like serial sections through the fern stele model, even when overall abundance remains relatively constant (Fig. 2). The moths we have sampled range from known migrants to species considered localised, but density distribution is always changing. The time-scale is, of course, short compared with vertebrates. Our conceptual model predicts that once a density element has been defined in space, its life-span is limited. Our field samples confirm this,

Fig. 1 Conceptual model for population anatomy based on Rostowzew's⁷³ drawing of the stelar structure of the adder's tongue fern. The stele represents the space-time reticulum created by the distribution of an arbitrary density level through time.



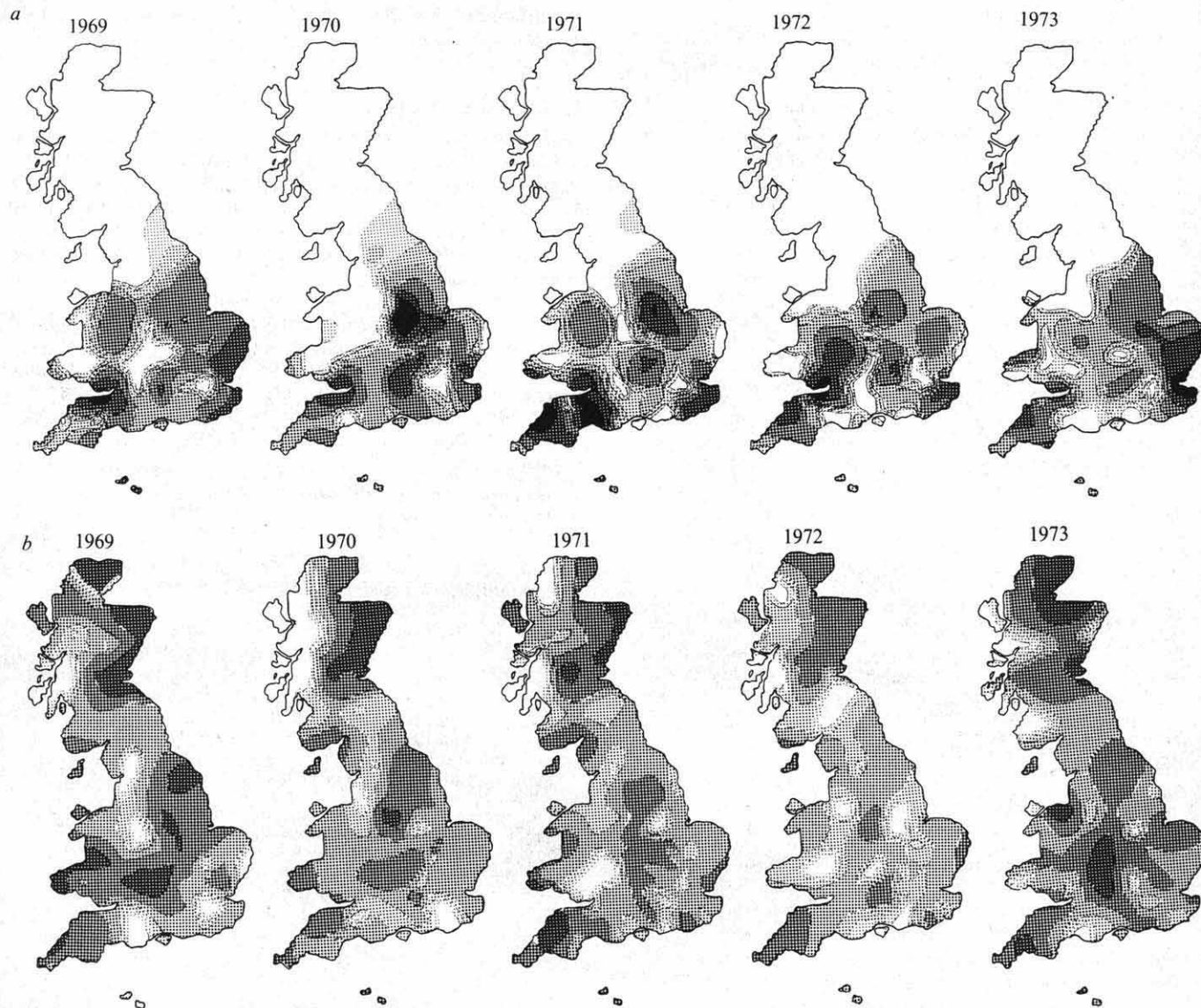


Fig. 2 Measured annual (1969-73) adult population density distribution of two moth species showing the changing patterns typical of the 623 species sampled by the Rothamsted Insect Survey⁴⁷. Each map is based on 62-88 sample points; there were inadequately distributed in Scotland, hence the poor definition there in (b). Density layers 0, 1-2, 3-9, 10-31, 32-99, 100-315, 316-999, individuals per sample unit. a, *Agrochola lychnidis* (Denis and Schiff.) a marginal species near its northern limit. b, *Xanthorhoe fluctuata* (L.) has a very stable total population. The maps are visualised as serial sections through the conceptual model in Fig. 1. In both instances the appearance and disappearance of 'holes' in the population is clearly visible so that all parts of the population are essentially 'marginal'.

and hence that the use of spatial averages, in fitness say³⁵, makes questionable assumptions about the nature of spatial distribution, for density can diminish locally to create what are usually regarded as marginal conditions^{49,50} even in the heart of a population (Fig. 2).

The individual's dilemma and the population consequence

In an environment changing through time and space, the most probable strategy for a new individual to adopt to survive and reproduce is not necessarily to stay to compete with its parents or congeners⁵¹ but may be to go elsewhere, to find an empty environmental hole to inhabit. Its ability to do this depends on its malleable migrant status. In a doomed habitat, one offspring capable of migrating at the right time may well have more survival value than a litter of more sedentary morphs⁴⁰. Thus the migrant status of offspring is an essential component of fitness controlling the production of grandchildren, which we take to be the relevant criterion. This point was originally made by Grinnell⁵² but he assumed population

centres to remain static and, like Gadgil⁵³ and others³⁵, regarded migration as compulsive for a genetically fixed proportion of the population. MacArthur⁵⁴ argued, legitimately, that such migration would be from an equilibrium population to a less hospitable place and involve a loss of fitness. Much environmental hostility which results in death or dispersal is transient, however, especially that due to biological restraints such as predation and disease or the loss of nesting sites or food supply. This creates vacant holes in the environment which subsequently recover their hospitality and are re-occupied by searching migrants so that population centres are mobile when seen at the appropriate time scale. These more fluid populations make MacArthur's argument unconvincing and, from the individual viewpoint, offer alternative courses of action. It was to an apparently social option that Hamilton⁵⁵ addressed his attention which he so graphically illustrated by his selfish frogs. The environmental menace was a predator and the choice considered was where to go within the herd so as "not to be nearest the snake". There is an additional option which Hamilton precluded, to abandon the herd and find a pond

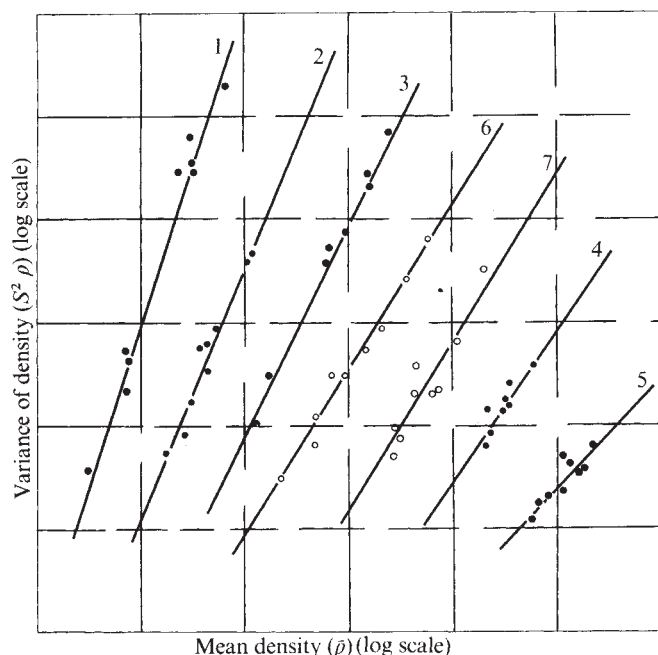


Fig. 3 The relation between variance of spatial disposition and annual mean density for five moth species from the same source as in Fig. 2, and two simulated quasi-stable populations. Each point is one years sample, equivalent to a map in Fig. 2. The fitted regression is $\log S_{pr}^2 = a + b \log \bar{p}$. The range of aggregatory response to overall population level differs widely between species but is highly consistent within species. 1, *Lycophotia varia* (Villers) ($a = 0.02$; $b = 3.02$). 2, *Xanthorhoe ferrugata* (Clerck) ($a = -0.29$; $b = 2.41$). 3, *Malacosoma neustria* (L.) ($a = 0.94$; $b = 1.98$). 4, *Xestia xanthographa* (Denis & Schiff) ($a = 1.16$; $b = 1.48$). 5, *Epirrhoe alternata* (Müller) ($a = 1.56$; $b = 1.04$). 6, From Fig. 5a; heterogeneous environment ($a = 1.38$; $b = 1.65$). 7, From Fig. 5b; homogeneous environment ($a = 1.35$; $b = 1.66$). Axes are staggered to save space. Grid is at $\times 10$ intervals. Symbols are (●) real samples, (○) from simulation.

without a snake. This individual choice creates recognisable spatial properties at population level which we suggest lead to population homeostasis without group selection^{56,57}.

A dynamic mechanism and a postulate

We would argue that all spatial dispositions can legitimately be regarded as resulting from the balance between two fundamental antithetical sets of behaviour always present between individuals. These are, repulsion behaviour, which results from the selection pressure for individuals to maximise their resources and hence to separate, and attraction behaviour, which results from the selection pressure to make the maximum use of available resources and hence to congregate wherever these resources are currently most abundant. The balance between these two conflicting behavioural tendencies operating on each individual determines its movements and hence the resulting spatial pattern of the population at any instant in time. It is the response of this balance to changing internal and external environmental conditions that, we suggest, constitutes the dynamic element in populations.

From this basis we have sought to bridge the gap between models and real data with a functional mechanism for the distributive processes in a population that would lead to the diffusion rates and spatial dispositions observed in nature.

The only hard spatial evidence we know of, that relates equally to populations of all kinds of organisms, is the statistical measure of fine-scale spatial disposition of individuals⁵⁸. Randomness is by definition atypical of the microdistribution of living organisms and one of us has previously shown empirically⁵⁹ that the spatial variance (S_p^2) characteristic of species, at a particular point in the environment, is proportional

to a fractional power of mean population density ($\bar{p}$) at that place;

$$S_p^2 = a\bar{p}^b, \quad (1)$$

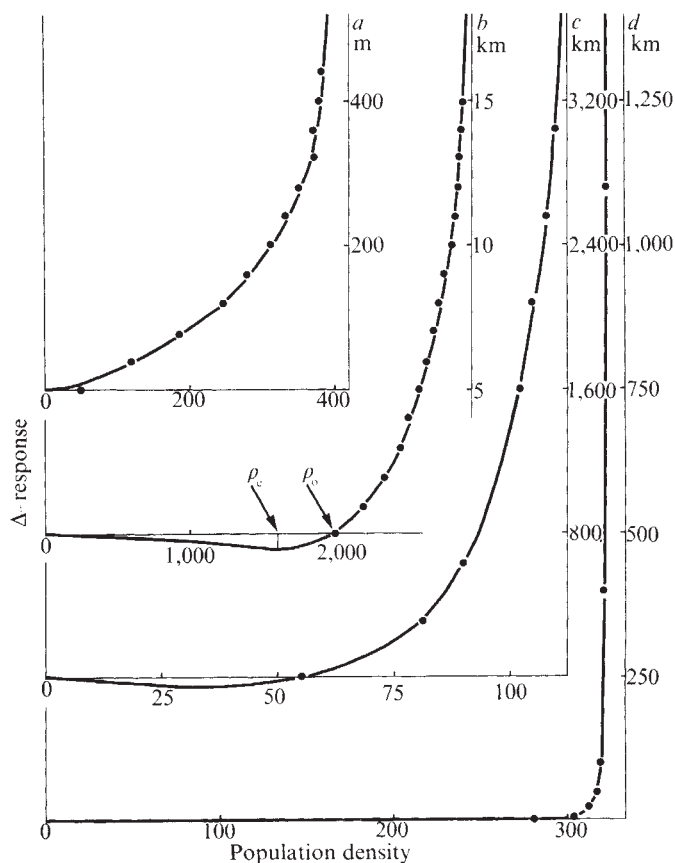
where b is a measure of the density dependence of aggregation. Our field monitoring now yields convincing evidence that macrodistribution, at least in Insecta, still has the same property of density-dependent aggregation when mean density relates to an area the size of Great Britain instead of a few square metres (Fig. 3). From this it is clear that, to return to the ferne stele model, successive stellar sections characterise the species spatial behaviour on a geographical as well as on a local scale.

The other relevant experimental evidence relates to rates of diffusion, or migration, of individuals from population concentrations, but for this there has yet been no generally accepted functional relation^{60,61}. Biological diffusion is no more random than spatial disposition, for it is the outcome of equally

Fig. 4 Δ -functions reflecting different life styles with superimposed density $\times$ distribution data from *a*, sedentary insect, *Drosophila pseudoobscura*; fluorescent dust recoveries at attractant traps in California²⁰. *b*, Social primate, man; census data from Sweden¹³. *c*, Migrant insect, monarch butterfly; wing-label recoveries in North America¹⁶. *d*, Territorial bird, blackbird; leg-ring recoveries in Great Britain²¹. Parameters

	ϵ	ρ_0	p	q
<i>a</i>	0.03	6	0.97	0.72
<i>b</i>	5.2	2000	7.3	6.7
<i>c</i>	11.0	54	7.2	1.1
<i>d</i>	7.3×10^{-22}	225	151	2.1

ρ_0 is the population density at which migratory and congregatory behaviour balance and no net movement ensues. At $\rho > \rho_0$ the population is over-dense and migratory behaviour predominates; at $\rho_c < \rho < \rho_0$ density is below par, congregatory behaviour predominates and leads to rapid recovery; at ρ_c , minimum Δ , congregatory behaviour is maximised; at $\rho < \rho_c$ spatial recovery is greatly impeded, T_R is prolonged and relies on high reproductive rate. Density units are arbitrary as in the original data. Densities below ρ_0 are difficult to measure and short-lived, hence the data points are inadequately represented.



sophisticated behaviour, and the problem has been to find a distance function common to such diverse organisms as, for example, the migrant monarch¹⁶ and the sedentary *Drosophila*²⁰, the territorial blackbird²¹ and social man¹³.

The working model does not deal with the underlying complexities of social behaviour⁶² and bizarre adornment associated with sex⁶³, which can be regarded largely as visible evidence of the congregatory behaviour, nor with the equally complex behaviour and locomotory mechanisms associated with migration²⁵, for they are too difficult to measure. It deals with the net displacement resulting from these. When the motion is centrifugal to the nearest population concentration it is here classified as 'migratory' to distinguish it from the random motion implied by 'dispersive'. Alternatively, when the motion is centripetal fulfilling the function of "gravitational attraction" in Skellam's⁶⁴ population concept, but missing from Lidicker's⁶⁵, it is here called 'congregatory' to distinguish its active motion from the resulting instantaneous static condition or aggregation. Migratory motion, like congregation, is overlaid by elaborate behaviour patterns and can be almost obscured by movements concerned with reproduction, fighting and feeding, especially daily and seasonal commuting in man and certain highly mobile and long-lived birds whose feeding and reproductive sites are far apart^{60,66}. Nor does it necessarily imply displacement right outside occupied territory, for much migration is internal. The displacement crucial to fitness is that between the birthplace of one generation and that of the next and it is the distribution of sexually mature adults as in Fig. 2 that is, therefore, relevant.

We postulate that the net centrifugal displacement of an individual (Δ_m) generated by migratory behaviour as defined above, is proportional to a fractional power of the population density (ρ) it experiences; $\Delta_m = G\rho^p$. So also is net centripetal displacement (Δ_c) due to congregatory (i.e. anti-migratory) behaviour; $\Delta_c = H\rho^q$. The exponents p and q are rate constants for density-dependent migration and congregation respectively which may be specific. G and H are constants of proportion for which we offer no biological interpretation.

If we adopt the sign convention that any tendency to increase the mean distance between individuals is positive while reduction in mean separation is negative, the intuitive sum of the two conflicting density-dependent behaviour sets is

$$\Delta = G\rho^p - H\rho^q \quad (2)$$

Equation (2) may be written in the parametric form

$$\Delta = \varepsilon \left[\left(\frac{\rho}{\rho_0} \right)^p - \left(\frac{\rho}{\rho_0} \right)^q \right] \quad (3)$$

where G and H are replaced by the biologically identifiable parameter ρ_0 , the density at which migration exactly balances congregation⁶⁷, and

$$\varepsilon = (H^p G^{-q})^{1/(p-q)} \quad (4)$$

which is a scale factor containing the dimensional units. Congregation (negative displacement) now occurs when $\rho < \rho_0$, and migration when $\rho > \rho_0$ (see Fig. 4). The condition $p < q$ cannot evolve because growing populations would continue to congregate indefinitely and condense into a point in space.

Simulation and interpretation

For simplicity in the primitive simulations presented here (Fig. 5), reproduction is by binary fission at random in space among those individuals reproductively mature, and at regular intervals in time. In an environment consisting of a permanent two-dimensional random mosaic of areas that are lethal, or tolerable but prevent reproductive maturation, or hospitable, the individuals of a founder population divide and each daughter organism responds, by movement, to the population density at the time of birth. This Δ -response is to the density

at a fixed population centre and is assumed to be radial. Mortality occurs only when movement ends in a hostile part of the environment. In practice the Δ -response may be to the individual's or to its parent's experience, to the distance from the nearest neighbour, or to number of social contacts, level of aggression or competition, or to other epideictic⁶⁶ or environmental cues relating to population density, and may not be radial. In a few generations population nuclei on hospitable areas reach a quasi-stable condition when migration balances natality (Fig. 5a). Satellite populations, repeating the process by responding to their own population centres, would ultimately occupy all the habitable areas.

Successive simulations with the environmental mosaic changing between generations (not shown here) generate additional movement and mortality. The resulting redistribution

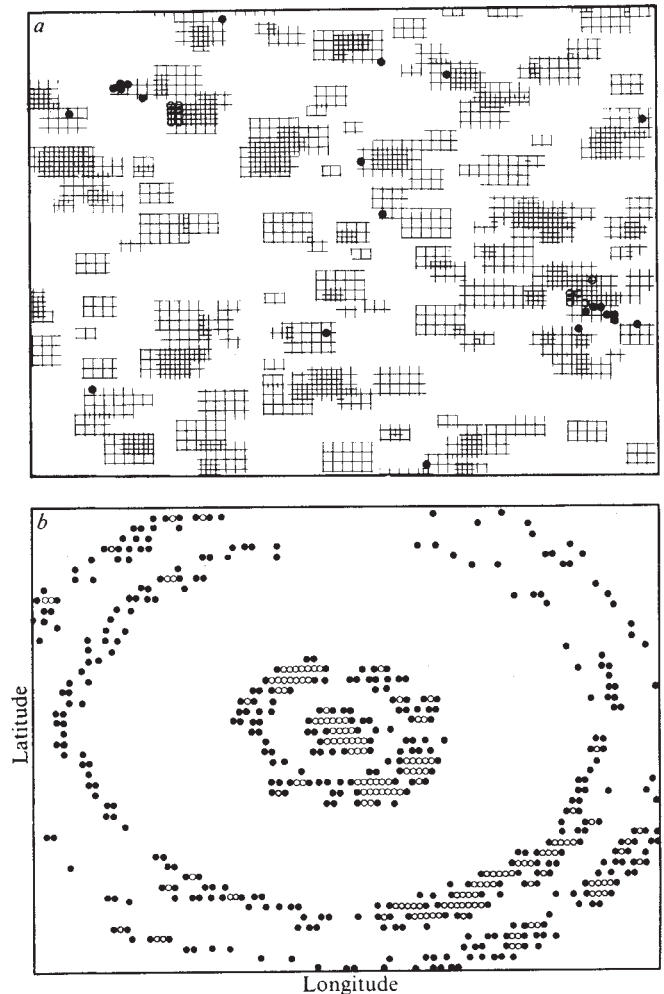


Fig. 5 *a*, Simulated population complex of a highly migrant species, something like an aphid, founded by three individuals and quasi-stable after five generations: Δ -response parameters, $\varepsilon = 0.333$; $\rho_0 = 40$; $p = 4$; $q = 2$, in an unchanging heterogeneous, mainly hostile, environment. Blank background is hostile, faint hatching tolerant but unproductive, denser hatching permits reproduction. At this stage two nuclei are reproducing and excess offspring migrate. Individuals on non-reproductive sites await an environmental change that will either release reproduction or kill them; their prospects depend on Southwood's¹⁶ τ/H ratio, currently being held in suspense by the models fixed environment. *b*, When the environment is homogeneous and the universe unlimited, density annuli radiate outwards from a point source in successive synchronised generations; parameters similar to (*a*). In this form the model describes the annular pattern of movement familiar, on a short time scale, as the radar ring angels⁷⁶ produced by the commuting flight of starlings in response to a daily cycle in the changing balance between feeding and flocking behaviours. The figures are distorted to conserve space. Hollow circles are occupied by more than one individual.

of population centres becomes a game of hide-and-seek, emulating the response of local population elements to predation, disease and other restraints, akin to Huffaker's survival by refuge⁶⁸. The net area population density now depends on the balance between rates of environmental change, and the movement and reproductive rates of the organism, which fails to use some resources because they are not discovered in time. Hence the population is stabilised below carrying capacity. Environmental change releases the reproductive potential of surplus individuals that wait in survival areas to mature if conditions improve, or to die if they worsen. The balance is set by the parameters in the Δ -function with respect to the environment into which further non-random elements are introduced because the Malthusian restraints are themselves biological and hence non-random^{86,69}. This now generates further local contraction⁷⁰ and small-scale geographical shifts of density like those clearly visible in successive years in Fig. 2.

The simplifying conditions adopted for simulation appear not to be restrictive. Age-dependent or facultative Δ -behaviour and sexual reproduction add complexity in the computing but without fundamental change in the outcome, so far as we can tell, for the resulting distance function appears to apply quite well to sexual animals with high levels of organisation (Fig. 4). Behaviour inherited or imprinted from the parents' or grandparents' experience or modified by experience throughout life, further complicates the computing but seems not to affect the outcome. Refinements of social and migratory behaviour, together with increase in size, minimise migratory risks by providing more accurate sensory information on which to make the choice. Again parental care and altruism add sophistication but seemingly make no fundamental change in the logic. Non-radial migration, by controlled orientation or navigation, or even environmentally assisted motion, affects individual migratory risks and hence proportionate mortality. It affects the ultimate spatial disposition only in large-scale geographical trends, because final settling place depends on the successful location of environmental holes, not just on navigation⁷¹. Death due solely to aging is a minor factor and all other causes are included in environmental adversity.

If each new recruit responds independently to density, a continuous generation model is formulated; synchronous response generates a discrete system as in the examples shown here.

The ecological results and the evolutionary consequences

Analysis of the spatial dispositions generated by simulation yields the power relation between variance and mean population density in equation (1) that is required by the field evidence (see Fig. 3). While the power relation could arise from mathematical models based on other functional mechanisms, this result suggests that our conceptual model for a balance between specific distributive behaviours is reasonable.

The Δ -function for individuals results in a density distributing process which generates a hollow frequency distribution of numbers against displacement in a population which matches the experimental evidence on dispersal for organisms as disparate in behaviour as *Drosophila* and man (see Fig. 4). Again, other functional mechanisms could generate appropriate distributions but no previous model has comprehended spatial behaviour over so wide a range of distances or organisms. We suggest that, if migration and congregation are seen in the collective sense used here, the dispersive behaviour of other animals and plants^{72,73} will be found to fit the pattern. The non-migratory condition when $\rho < \rho_0$ is perhaps most readily recognised in island life where ρ_0 is high due, for example in insects, to flight-inhibiting behaviour and even morphological adaptation such as brachyptery.

The only biological premises involved are that an organism can move at some stage in its life history before reproduction and can detect directly or indirectly, by any means, including those listed by Wynne-Edwards⁶⁶ and Wilson⁶², the presence

of another of its own kind and hence can respond to it. Reproductive success then often lies in the avoidance of overt competition by movement and goes to the individual with the best Δ -adaptation, rather than to the more superficially successful competitor, and so provides an essential^{70,74} counterbalance to excessive competitiveness.

As with other population models, migration and mortality can in some respects be equated. The emphasis is here shifted from the key factors that cause death to the place where they operate, because this reflects the real space hostility pattern of the total environment, including other organisms, and to the subsequent replacement of individuals by movement as well as by birth. Thus the return time for populations displaced from equilibrium, T_R (ref. 8), can be short-circuited by migration. A demon with perfectly developed Δ -response and ability to locate environmental holes could survive almost any catastrophe. If survival is thus seen as a spatial exercise in eluding adversity, evolution depends on learning the geometry, not just of Hamilton's selfish herd⁶⁵, but of the changing Darwinian landscape of which the herd is a part.

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Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes

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The culture lifetime of epidermal cells of newborn humans is increased from 50 to 150 generations by adding to the medium epidermal growth factor, a polypeptide mitogen. EGF seems to delay senescence of the cells by maintaining them in a state further removed from terminal differentiation. This effect is revealed by a greater ability of the cells to survive subculture and initiate new colonies, but not necessarily by an increased growth rate.

MAMMALIAN epidermis is composed principally of a single cell type, the keratinocyte, in various stages of differentiation. The basal layer, separated by a basement membrane from the fibroblasts of the underlying dermis, contains the dividing keratinocytes. These give rise to progeny, some of which no longer divide but move outward from the basal layer, terminally differentiate, and eventually are shed from the surface.

In cell culture conditions, keratinocytes depend on the support of fibroblasts in order to initiate colony formation^{1,2}. When human diploid epidermal keratinocytes obtained from foreskin of newborns are inoculated together with lethally irradiated 3T3 cells, the keratinocytes grow from single cells into macroscopic colonies. Colonies can be disaggregated to single cells with trypsin and EDTA and the cells subcultured with fresh irradiated 3T3 cells. A fraction of the keratinocytes undergoes terminal differentiation even in growing colonies; these cells leave the basal layer, increase in size, and develop an envelope resistant to detergents and reducing agents³. At least partly because of this terminal differentiation, colony-forming efficiency on transfer is lower than that of fibroblasts. Keratinocytes derived from foreskin of newborn humans were cultivated serially through a total of about 50 cell generations but those of older donors had a reduced culture lifetime (ref. 2 and unpublished observations). This behaviour resembles that of cultivated human fibroblasts, whose restricted culture lifetime has suggested an analogy to the process of ageing⁴.

Epidermal growth factor (EGF), a polypeptide with molecular weight 6045 and a known amino acid sequence⁵ was first isolated from the male mouse submaxillary gland by S. Cohen, and found to enhance growth and maturation of epidermis in newborn mice^{6,7}, and in organ cultures of epithelial tissues^{8,9,10,11}. It has more recently been found to be an extremely potent mitogen for fibroblasts^{12,13,14}, and for some other cell types (refs 15, 16, and R. Holley, personal communication). Most cells bind EGF with a K_m of $\sim 1 \text{ ng ml}^{-1}$, a concentration similar to that present in mammalian plasma¹⁷.

We describe here experiments on the effect of mouse EGF on cultivated epidermal keratinocytes derived from foreskin of newborn infants. The culture methods, including the use of irradiated 3T3 cells, have been described earlier^{1,2,3}. For the first experiments, the EGF was kindly donated by Dr Cohen; later experiments were carried out using EGF purified by Dr T.-T. Sun according to Savage and Cohen¹⁸. The effects we describe have a bearing on the general problem of the lifetime of serially cultivated cells, and on possible relations between this phenomenon, ageing and terminal differentiation.

Effect of EGF on colony expansion

Culture dishes (50 mm) were inoculated with 5×10^3 epidermal keratinocytes and one-third the confluent density of lethally irradiated 3T3 cells (x3T3). As EGF was found

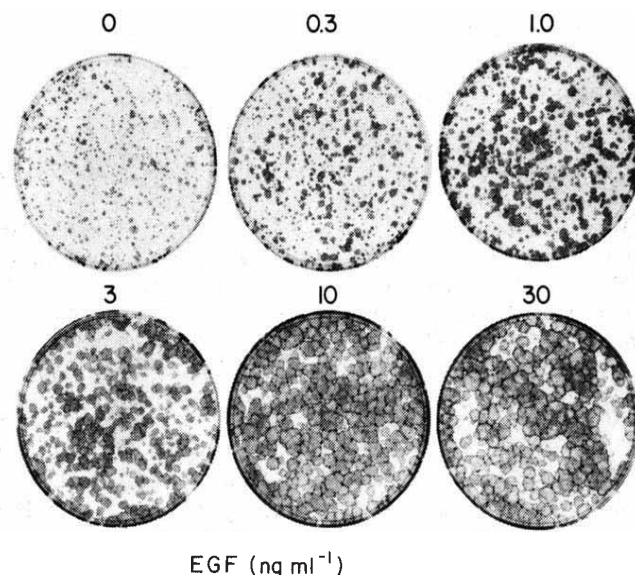


Fig. 1 Effect of EGF on the morphology and size of growing keratinocyte colonies. Dishes (60 mm) were inoculated with 5×10^3 keratinocytes of strain N at their 43rd culture generation and the Dulbecco-Vogt modification of Eagle's medium supplemented with 20% fetal calf serum and hydrocortisone, $0.4 \mu\text{g ml}^{-1}$. EGF was added at various concentrations beginning on d 4. Cultures were fixed on d 13 and stained with Rhodanile blue¹. Colonies grown in the presence of EGF are larger, but also thinner and the intensity of their red staining by Rhodanile blue is reduced. At higher concentrations of EGF, the colonies sometimes acquired a shredded appearance, and growth was retarded.

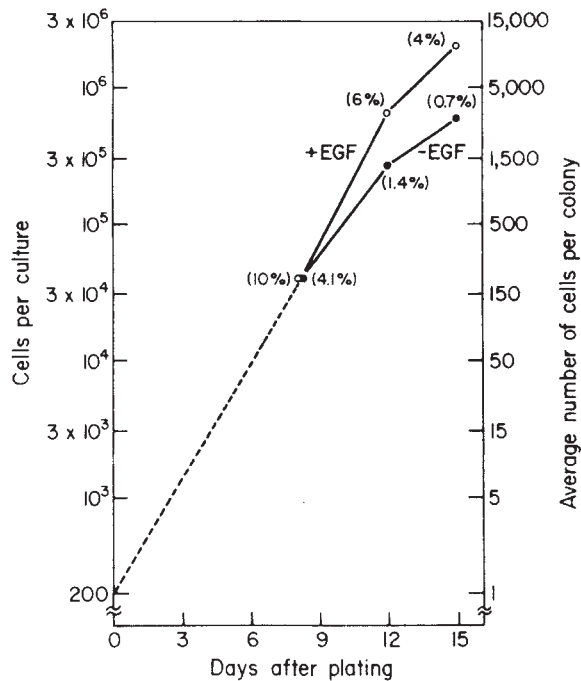


Fig. 2 Effect of EGF on growth rate and colony-forming ability of cultured human epidermal keratinocytes. Strain M. keratinocytes, at their 20th generation in culture, were plated at 1.9×10^4 cells (200 colony-forming units) per 60 mm dish, together with lethally irradiated 3T3 cells. Beginning at the first medium change 5 d after plating, EGF was added at 30 ng ml^{-1} to half of the cultures. Cultures were refed every 3–4 d thereafter. On d 8, d 12, and d 15 after plating, the x3T3 cells of a culture were removed with EDTA; the epidermal colonies were then dispersed to single cells with a mixture of trypsin and EDTA and counted in a haemocytometer. Aliquots were replated with x3T3 to determine colony-forming ability.

○, Cultures to which EGF was added beginning on the 5th day after inoculation; ●, no EGF. Values in parentheses give the colony-forming ability of the cells upon subculture. Cells growing in the presence of EGF have a higher colony forming ability and maintain exponential growth for a longer period.

to reduce colony-forming efficiency slightly when added at the time of inoculation, it was routinely added to cultures at the time of the first medium change, 3–5 days later. Figure 1 shows the appearance of colonies fixed and stained on the 13th day after inoculation. Colonies growing in the presence of EGF were larger, less stratified, and stained less intensely red with Rhodanile blue. These effects were evident at EGF concentrations as low as 0.3 ng ml^{-1} and increased in magnitude up to concentrations of 10 or 30 ng ml^{-1} . Part of the increase in colony size is due to enhancement of growth and part to the spreading and flattening of the cells. When EGF is added to cultures of large colonies grown in the absence of EGF, change in

colony morphology is often evident as soon as 24 h later.

In contrast to the effects of EGF, fibroblast growth factor (FGF), kindly provided by Dr Denis Gospodarowicz, produced no changes in colony morphology or growth at concentrations of $1\text{--}100 \text{ ng ml}^{-1}$, in keeping with its activity as a growth factor only for mesenchymal cells¹⁸.

Effect of EGF on the growth rate and colony-forming efficiency of keratinocytes

Early passage human keratinocytes were plated at low density and grown in the presence or absence of EGF at 30 ng ml^{-1} . Figure 2 shows that while the colonies were still small, they did not grow more rapidly in the presence of EGF, but as the colony size increased, exponential growth was maintained longer and by the end of the experiment (d 15) the number of cells was about four times greater in the presence of EGF. The average doubling time for the cells while in exponential growth was about 24 h, both in the presence and absence of EGF.

Some of the cells harvested for each count were replated with fresh x3T3 cells to determine their ability to start new colonies (Fig. 2, values in parentheses). The colony-forming ability of the cells fell as colonies grew larger and as their average growth rate declined, but was always greater for the cells derived from cultures grown in the presence of EGF. The difference was small at first but became progressively larger as the colonies became larger. At the end of the experiment (d 15) the difference was about sixfold.

The effect of EGF on small colonies was more closely examined by using two other strains of newborn keratinocytes. Cultures were plated with 10^5 cells of strain N or strain K, previously grown through one subculture in the absence of EGF. EGF was added to some cultures 2 d after inoculation and the growth and colony-forming ability of the cells of the two sets of cultures were compared during the following 4 d. EGF had no significant effect on growth rate (Table 1), but increased the colony-forming efficiency of the cells by 50% within 2 d of its addition and by 60% to twofold within 4 d.

These experiments suggest that the effect of EGF on the growth rate of large colonies is due to its ability to oppose inhibitory influences that begin when the cells become crowded. There are several possible ways by which this effect might be brought about, but all would have to alter either the generation time or the relative magnitude of the dividing fraction. (These alternatives are not completely independent, since an increase in the growth rate of the dividing fraction would, if the time required for terminal differentiation were unchanged, lead to an increase in the relative size of the dividing fraction.)

The fact that EGF increases colony-forming ability before the growth rate is detectably increased indicates that, even while the magnitude of the dividing fraction is unaltered, there is a change in the state of the dividing cells

Table 1 Effect of EGF on the cells of small keratinocyte colonies

Strain	Days after Inoculation	Cells per culture ($\times 10^{-3}$)		Colony forming efficiency on subculture (%)	
		– EGF	+ EGF	EGF	– EGF
N (23 gens.)	2	0.74	—	9.7	—
	4	2.6	2.1	13.3	19.5
	6	11.0	9.8	7.0	11.5
K (25 gens.)	2	0.56	—	5.2	—
	4	3.4	2.7	7.6	11.3
	6	16.0	13.0	3.5	7.2

Cultures were inoculated with 10^5 keratinocytes and x3T3 cells on day zero. Cultures were refed on d 2 and EGF was added to 10 ng ml^{-1} . At intervals the 3T3 cells were removed from a culture with EDTA and the keratinocytes were harvested in trypsin and EDTA. After counting the cells, some were reinoculated with x3T3 cells to determine their colony forming ability. Values are means ($\pm 15\%$) obtained from duplicate cultures.

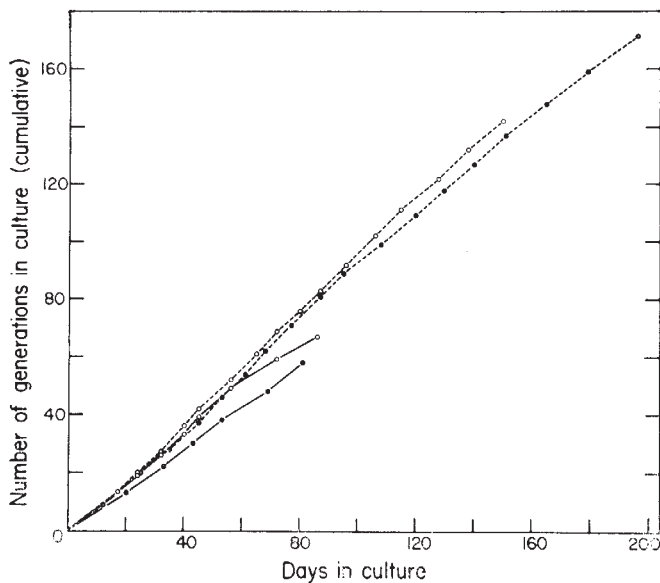


Fig. 3 Effect of EGF on the culture lifetime of human epidermal keratinocytes. The number of cell generations was calculated from the number of colonies formed and the number of cells harvested at each transfer. Each point represents a single transfer. The last point was obtained from the harvest of the last culture in which colonies formed. The average growth rate is given by the slope. ---, Cultures grown in the presence of EGF; —, controls grown on the absence of EGF. ●, Strain HFE; ○, strain E.

better enabling them to survive disaggregation and form new colonies. In order to have this effect, it was found that EGF must interact with cells able to proceed through the growth cycle. For example, if keratinocytes were prevented from growing by suspending them in medium stabilised with methyl cellulose, they lost their colony forming ability with a half life of 3–4 h, and later acquired markers characteristic of the terminally differentiated state. This extremely rapid loss of colony forming ability by keratinocytes, so different from the behaviour of fibroblasts, did not seem to be affected by the presence of EGF, or by growing the cells in the presence of EGF immediately before inoculating them into suspension cultures. Clearly then, keratinocytes can respond to EGF only if they are able to grow. Further data on this effect of EGF were obtained from the studies of serially cultivated cells described below.

Effect of EGF on the culture lifetime of keratinocytes

As reported earlier, the culture lifetime of newborn human epidermal keratinocytes in the absence of EGF did not exceed 50 cell generations². As cultures maintained a higher proportion of colony forming cells in the presence of EGF, we examined the effect of EGF on the culture lifetime. In this experiment, contaminating dermal fibroblasts were selectively removed³ during the first two subcultures, and therefore never overgrew the keratinocytes in later subcultures. The cultures were always transferred while the cells were in exponential growth. For these reasons the values for culture lifetime were more reproducible than those obtained in conditions used earlier².

Two independent human keratinocyte strains, HFE and E, were serially transferred by inoculating 2×10^4 – 2×10^5 cells per 60 mm dish and subculturing 7–11 days later. EGF was added beginning with the first refeeding at 3–5 days after each inoculation. The colony forming efficiency of the cells at each subculture was determined by plating 3×10^3 – 3×10^4 keratinocytes and counting the number of colonies produced. All inoculations were made together

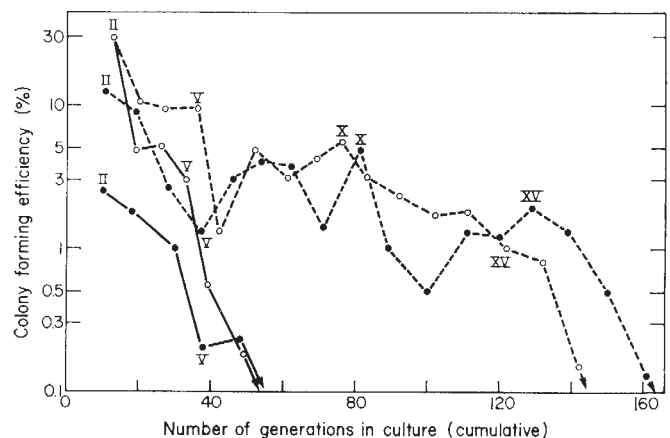
with the standard density of irradiated 3T3 cells. The number of generations in each subculture was derived by relating the number of colonies (assuming each to be initiated by a single cell) to the total number of cells harvested. The number of generations so calculated is an average for the descendants of each colony-forming cell; but because of terminal differentiation in growing colonies, the dividing fraction undergoes more than the average number of doublings.

The maximum culture lifetime, as found earlier, of the two keratinocyte strains derived from newborns, and grown in the absence of EGF, was 6–7 transfers and about 50 cell generations. Addition of EGF to the medium increased the culture lifetime to 17–18 transfers and over 140 cell generations (Fig. 3) but did not significantly affect growth rate (the slope of the lines). Also, the growth rate declined very little on serial cultivation in the presence or absence of EGF until two transfers from the end of the life of the cultures. Thus the effect on culture lifetime does not depend on a change in growth rate.

When colony formation was examined at each transfer, however, large differences were apparent (Fig. 4). In the absence of EGF, the colony forming efficiency during the early transfers was in the range of 2–10%. At each transfer colony formation declined, and when it dropped below 0.1% (by about 50 generations), it was not possible to obtain growth at the next transfer. Colony formation by cells grown in the presence of EGF was higher from the very beginning, and although it declined with serial transfer, the rate of decline was much less than in the absence of EGF. The efficiency was still about 1% after 16 transfers, when both strains had reached 130 cell generations and were close to the end of their culture lifetime. An orcein stained chromosome preparation of strain HFE at 140 generations revealed a diploid chromosome number and no obvious abnormalities.

The number of keratinocytes which can be generated in culture through serial transfer depends not only on the number of cell generations the population can be grown but also on the plating efficiency at each transfer. In the absence of EGF, the possible expansion in culture of the keratinocyte population of newborns does not exceed a factor of 10^6 . Since EGF increases both lifetime and plating efficiency, the corresponding value exceeds 10^{16} . Clearly, if large numbers of culture grown keratinocytes have any practical use such as for grafting, EGF would be valuable for the cultivation.

Fig. 4 The effect of EGF on colony formation during the culture, lifetime of human epidermal keratinocytes. ---, Cultures grown in the presence of EGF; —, cultures grown in the absence of EGF. ●, Strain HFE; ○, strain E. Roman numerals give transfer numbers. Each value for colony forming efficiency was derived by inoculating cells at 2 or 3 dilutions, so as to obtain at least two dishes containing 50–300 colonies.



Significance of the restricted lifetime of cultured mammalian cells

The existence of a reproducible lifetime of cultured human diploid fibroblasts²⁰ has suggested an analogy to the process of ageing and has led to many studies on the phenomenon, as well as to theories of its basis^{4,21}. Although a reproducible lifetime can be obtained for fibroblasts under standard conditions, the lifetime can be increased by improving the culture conditions^{22,23,24}. This would not be expected if the lifetime were limited by a fixed rate of mutational errors or errors in DNA replication. The dependence of the culture lifetime of diploid cells on the culture conditions is shown more clearly by keratinocytes, for we have succeeded in tripling the culture lifetime of this cell type by simply including EGF in the medium.

Although the culture lifetime of human keratinocytes in the presence of EGF exceeds considerably that of human fibroblasts under the best conditions, it is still far below what should be expected from the doubling time of the basal cell population *in vivo*². Our method of calculating culture lifetime does not consider that some cells terminally differentiate in growing colonies and are therefore removed from the proliferating pool. Cells remaining in the pool therefore undergo more divisions than are calculated from the total cell yield per colony. Although we cannot correct for this factor, it seems likely that at least a small part of the discrepancy between culture lifetime and *in vivo* lifetime of the keratinocytes may be explained in this way.

It has been suggested²⁵ that restriction of the culture lifetime of fibroblasts and the depletion of colony forming smooth muscle cells of older animals are due to terminal differentiation, but no suitable marker exists to define a terminally differentiated state in these cell types. Haematopoietic cell populations, whose proliferative behaviour is usually described in terms of stem cell kinetics²⁶, show a decline in the number of colony forming cells during serial transplantation *in vivo*²⁷, a process apparently related to the increasing differentiation of the progeny.

For the cultured keratinocyte, we have ample evidence of ultimate terminal differentiation. In addition to loss of ability to form colonies, the process includes a large increase in cell size, the formation of cornified cell envelopes resistant to detergents³, and the later development of squames whose nuclei and cytoplasmic organelles are destroyed, resulting in a final state resembling cells of the stratum corneum. Details of this differentiation will be described elsewhere; of importance here is that when

growth of cultured keratinocytes is arrested, they enter this state of terminal differentiation. This is true at any stage of their culture life.

We have shown that multiplying keratinocytes can exist in different states related to their distance from obligatory terminal differentiation. That these states are revealed by the colony forming ability of the cells but not always by their growth rate is shown by two types of experiments. First, under conditions in which EGF did not increase growth rate, it increased the ability of the cells to form new colonies. Second, there was little or no decline in growth rate on serial transfer and no difference in growth rate between cultures in the presence or absence of EGF until close to the end of their culture lifetime; in contrast, colony forming ability declined to one-tenth or one-twentieth of the initial values before evident changes in growth rate, and EGF reduced this rate of loss almost from the very beginning. Subculture evidently selects against those hitherto dividing cells too close to terminal differentiation to re-enter the division cycle after transfer. We suggest that EGF extends the culture life of keratinocytes by increasing the distance of the multiplying cells from obligatory terminal differentiation.

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letters to nature

Cosmic-ray electrons and galactic radio emission—a conflict?

MANY attempts have been made in the past to deduce information of astrophysical importance from a study of the galactic non-thermal radio continuum in relation to cosmic-ray electrons observed in the neighbourhood of the Earth (refs 1 and 2 and references therein). These investigations were carried out using the cosmic ray electron data obtained from a single experiment or by making use of an average spectrum derived from the world data, although it is known that the flux values observed by different

experimenters in any energy band differ by as much as a factor of 4. This in turn has led to conflicting conclusions to be drawn from the analyses of different authors. In this paper, we present a different approach for analysing the observational data based on arguments of internal consistency between each measured electron spectrum and the magnetic field strength and the dimension of the radio-emitting region required to explain the radio observations. Such an analysis makes it possible to highlight the inconsistencies associated with some of the electron measurements and permits certain inferences of cosmic ray and astrophysical interest.

We will refer first to the spectral shape of the cosmic-ray electrons expected from radio observations. It is found from the latest compilation of the radio intensity, $I(\nu)$, by A. S. Webster (personal

communication) between 10 and 8,000 MHz in the direction of the anticentre, that the spectrum above 300 MHz can be well represented by a simple power law of the type $I(\nu) \propto \nu^{-\alpha}$ with $\alpha = 0.79 \pm 0.08$. On the basis of the theory of synchrotron radiation, this implies that the energy spectrum of radio-emitting electrons in interstellar space is also a simple power law of the type $j(E) = AE^{-\gamma}$, where $\gamma = 2.58 \pm 0.16$ in the energy range of roughly 4–20 GeV for a typical magnetic field strength of $4 \mu\text{G}$ perpendicular to the line of sight; here $j(E)$ expressed in particles $\text{M}^{-2} \text{sr}^{-1} \text{s}^{-1} \text{GeV}^{-1}$ and A is a constant. Similarly, the radio spectrum in the direction of the halo minimum has the same spectral index between 200 MHz and 8,000 MHz. From an examination of the radio spectra from these two directions, it is inferred that the simple power law for the radio-emitting electrons should extend to at least 25 GeV and perhaps, far beyond. It is known from observations on cosmic-ray electrons above 5 GeV that they are not affected by solar modulation and, therefore, we expect that the energy spectrum of electrons observed in the neighbourhood of the Earth should also have a spectral index $\gamma = 2.58 \pm 0.16$ in the energy range between about 5 GeV and at least 25 GeV.

Table 1 summarises the experimental data on cosmic ray electron spectra determined by various experimental groups^{3–15}. In this table, we have listed the experiments in ascending order of their departure in standard deviation from the expected spectral index of 2.58 ± 0.16 . We have also examined the spectral index in the limited energy range between 5 GeV and 30 GeV, for those experiments which cover a wider energy range, and have found it to be the same within one standard deviation of the value given in the table. It is quite encouraging to notice that most of the experimental values of γ are consistent with the radio data within about 2σ . However, the measurements of Silverberg *et al.*¹⁴ and Meegan and Earl¹⁵ show departures by about 3σ and 4σ respectively and are thus in conflict with the radio data.

Table 1 Observed energy spectrum of cosmic-ray electrons above 5 GeV

Reference	Energy range (GeV)	A	γ	Departure (s.d.)
Marsden <i>et al.</i> ³	5–15	287	$2.77 \pm 0.42^*$	0.4
Anand <i>et al.</i> ⁴	12–750	113	2.69 ± 0.10	0.6
Scheepmaker and Tanaka ⁵	5–290	127	2.70 ± 0.10	0.6
Zatsepin ⁶	7–500	95	2.70 ± 0.05	0.7
Müller and Meyer ⁷	30–900	51	2.75 ± 0.10	0.9
Agrinier <i>et al.</i> ⁸	8–100	24	2.30 ± 0.20	1.1
Buffington <i>et al.</i> ⁹	5–63	105	2.80 ± 0.10	1.2
Freier <i>et al.</i> ¹⁰	8–100	105	3.00 ± 0.20	1.6
Webber and Rochstroh ¹¹	3–20	100	3.00 ± 0.20	1.6
Matsuo <i>et al.</i> ¹²	40–800	377	3.20 ± 0.30	1.8
Hovestadt <i>et al.</i> ¹³	5–22	204	$2.97 \pm 0.13^*$	1.9
Silverberg <i>et al.</i> ¹⁴	10–220	430	3.10 ± 0.08	3.2
Meegan and Earl ¹⁵	6–64	800	3.40 ± 0.10	4.3

*In these cases A and γ have been determined by us from the data.

We next examine the implications of the absolute intensity of cosmic ray electrons measured by various groups on the basis of the observed radio flux at 1,400 MHz toward the anticentre, which is found to be about $6.7 \times 10^{-22} \text{ W } (\text{M}^{-2} \text{sr}^{-1} \text{Hz}^{-1})$, after normalising the spectrum given by Webster (personal communication) at 81.5 MHz using Purton's survey¹⁶. This value of the frequency was chosen in order to avoid sampling of electrons below 5 GeV, for the wide range of magnetic field strength used in the analysis. We then calculated for each electron spectrum given in Table 1 the extent of the radio-emitting volume for different mean field strengths perpendicular to the line of sight needed to account for the radio flux at 1,400 MHz, by assuming that the cosmic ray electrons and the magnetic field are homogeneously distributed throughout the emitting region. We also assumed that, for those measurements in which the minimum energy given in Table 1 is much larger than 5 GeV, the same electron spectra

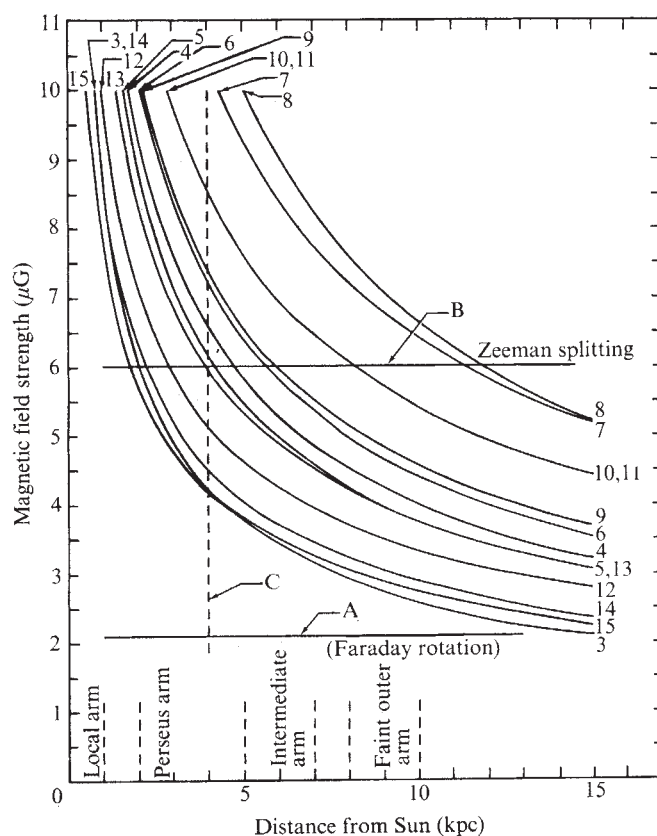


Fig. 1 The calculated mean magnetic field is plotted against the extent of the radio-emitting region toward the anticentre for various cosmic-ray electron measurements listed in Table 1 (details in text).

extend down to this energy. In Fig. 1, we have plotted the calculated magnetic field strength as a function of the extent of the radio-emitting region toward the anticentre for all the electron measurements listed in Table 1. We indicate in this figure the approximate position of various spiral arms, the longitudinal component of the magnetic field (line A) in the local arm from Faraday rotation measurements¹⁷, and a reasonable upper limit to the field strength (line B) deduced by us on the basis of the marginal detection of $2.1 \pm 2.0 \mu\text{G}$ for the ambient field in the local arm using the Zeeman splitting of 21-cm hydrogen line¹⁸.

One observes from Fig. 1 that all the calculated curves fall above line A, thereby, demonstrating that either the magnetic field derived from Faraday rotation measurements is an underestimate or that the intensity of radio emitting electrons in the Galaxy is larger than that observed by any group listed in Table 1. However, gamma-ray observations in the energy region above 100 MeV¹⁹ show that the interstellar intensity of nucleonic components of the cosmic rays in the few GeV region is not very different from that observed near the Earth. Therefore, we conclude from this argument that the cosmic ray electron measurements set a lower bound to the mean magnetic field of $2 \mu\text{G}$ for the interstellar space and that Faraday rotation measurements are indicative of a lower limit to the local magnetic field in the Galaxy.

It is generally recognised that the radio-emitting region toward the anticentre does not extend much beyond the Perseus arm, because the Intermediate arm itself is very faint in 21-cm observations having a gas density less than one-fifth of that of the Perseus arm²⁰. We have, thus, drawn a line C in Fig. 1 at 4 kpc, as the most probable equivalent dimension of the radio-emitting region with a mean emissivity similar to that in the local arm. The intercepts of this line with the calculated curves indicate that the required field strength varies from about $4 \mu\text{G}$ to $11.5 \mu\text{G}$, which is two to six times higher than that deduced from Faraday rotation measurements. In particular, the measurements of Freier *et al.*¹⁰, Webber and Rochstroh¹¹, Müller and Meyer⁷, and Agrinier *et*

al.⁸, give values $\geq 8.5 \mu\text{G}$, which are much higher than even the upper limit set from the Zeeman splitting measurement (line B). In other words, these measurements would imply (from their intercepts with line B) an equivalent emitting region of at least 8 kpc toward the anticentre, which is difficult to accept regardless of the uncertainties associated with the positions of spiral arms in the outer parts of the Galaxy. Such large magnetic fields also require very high gas density in order to be contained in the galactic disk²¹.

In conclusion, we have shown that (1) the observed spectral index of the radio continuum in the Galaxy is in conflict with some of the cosmic-ray electron measurements^{14,15}; (2) the absolute intensities of cosmic-ray electrons measured by some of the experimenters^{7,8,10,11} are so low that they cannot be reconciled either with the interstellar magnetic field limits or with the extent of the galactic disk toward the anticentre; and (3) it is likely that the field strength derived from Faraday rotation measurements give only a lower limit to the local magnetic field in the Galaxy.

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Computation of periods of acoustical oscillations of the Sun

DISCOVERY of the regular pulsations of the Sun was reported in 1975–1976 by three groups of investigators^{1–3}. It has been difficult to identify these oscillations, particularly the very stable 2 h 40 min one^{1,2,4}. Having had experience in calculations of the free oscillations of gaseous–liquid Jupiter and Saturn⁵, we decided to compute the periods of some acoustical modes for one of the complete solar models. We chose the Abraham and Iben⁶ Model I as a model of the interior, and a model of the convective zone from Spruit⁷ which had a depth of 198,00 km; an empirical model HRSA⁸ was taken as a model of the solar atmosphere.

The equations of small adiabatic oscillations and boundary conditions which we used are the same as those used for giant planets⁵ and similar to those used since 1959 for the free oscillations of the Earth⁹. The equations are also similar to those usually used in stellar astrophysics^{10,11}, but boundary conditions have a slightly different form. Our method of computation¹² is similar to that used for the giant planets. The only

Table 1 Periods jT_{sn} of free oscillations of the Sun

j/n	Period (min)						
	0	2	3	4	5	6	7
0	48	191	136	110	93.6	82.6	74.6
1	31	44	41	38.5	36.4	34.5	
2	23	30	28	27	25.5	24.5	
3	18	23	21.3	20.2	20.0		
4	—	18					
5	13						
6	11						

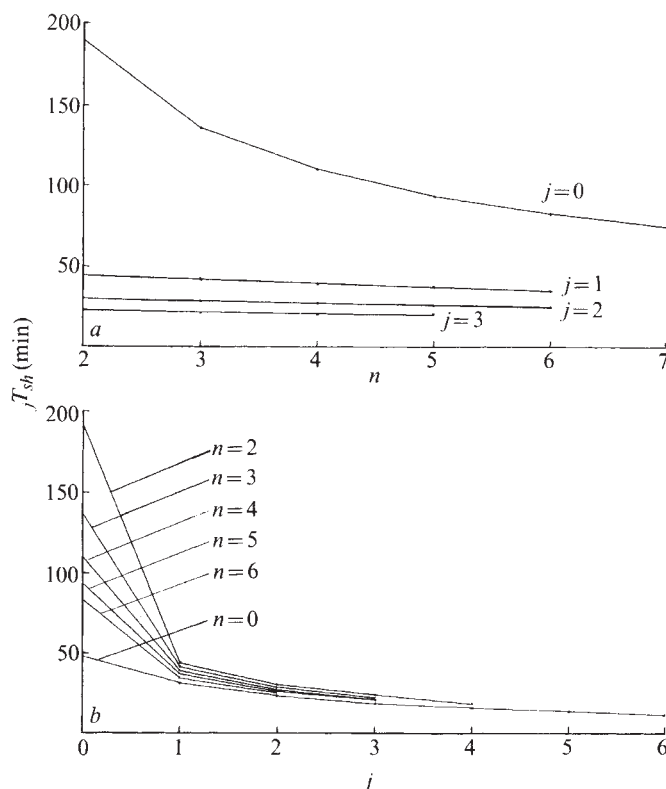
difference is that here we have not used an analytical start from the centre when the equations were numerically integrated. We always started with orthogonal vectors at some distance from the centre. This technique, calling for special attention when applied, proved itself to be correct for Jupiter and Saturn as well as for the Earth. Such a technique permits the calculations of acoustical modes only, separately from gravity modes, because gravity modes are formed near the centre.

We report our results in Table 1 and Figs 1 and 2. The calculated periods of free oscillations are presented in Table 1. jT_{sn} denotes the period of the j th overtone of a spheroidal mode characterised by a spherical harmonic of degree n . The classification of radial modes ($n = 0$) on to overtones is that accepted for the oscillations of the Earth. The accuracy of computations is about 1 min for fundamental radial and quadrupole ($n = 2$) modes; the accuracy improves when either n or j increases.

Figure 1 shows the periods jT_{sn} as functions of both n and j .

The vector of displacements of a material point is characterised by its radial and tangential components. Figure 2 shows the tangential component V as a function of indimensional radius $\beta = r/R_{\odot}$ for the fundamental mode and the first overtones of quadrupole oscillations. The application of tangential component is more visible because of very strong attenuation of radial component with depth. For overtones $V(\beta)$ has an oscillating character; index j determines the number of nodes.

Fig. 1 The periods jT_{sn} : a, as functions of n , for the fundamental mode $j = 0$ and first overtones $j = 1-3$; b, as functions of j , for radial modes $n = 0$ and first spheroidal modes $n = 2-6$.



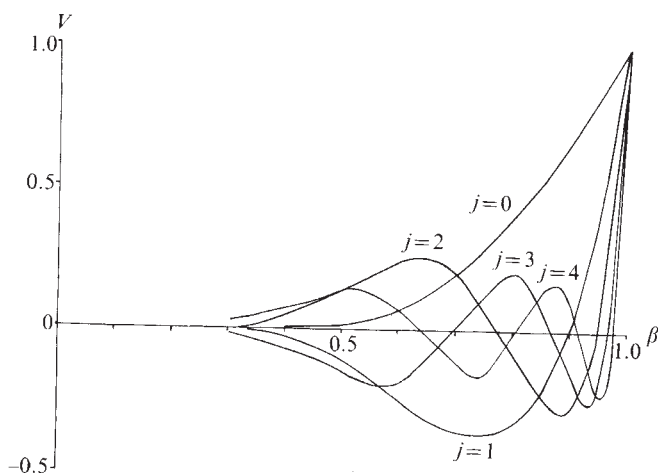


Fig. 2 The tangential component of displacements $V(\beta)$, $\beta = r/R_{\odot}$, for the fundamental mode and first overtones of quadrupole oscillations.

In order to invert the procedure to improve the solar models, the first step is to identify precisely the observed oscillations. The comparison of the observed periods of oscillations of the Sun¹⁻³ with the computed periods in Table 1 makes it possible to assume that the mode with a period of 2 h 40 min (refs 1, 2) is the fundamental quadrupole mode, the mode with a period of 48 min (ref. 3) is the fundamental radial mode; the 40-min period² corresponds to the first quadrupole overtone, the 29-min period² corresponds either to the second quadrupole overtone or to the first radial overtone.

There are some discrepancies between our results and those of Christensen-Dalsgaard and Gough⁵, and so there is another identification of the observed periods of the oscillations of the Sun. We think that the cause may be in the model. We could not examine the discrepancy in detail because we had not got the basic model for which the periods in ref. 5 were computed.

Recent calculations indicate that more accurate computations of the eigenfunctions in the inner regions may shift the periods slightly, without influencing the identification proposed.

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On presolar meteoritic sulphides

DISCOVERIES of isotopic anomalies in meteorites¹⁻⁴ inspired those same research groups to postulate nucleosynthetic inhomogeneities that were somehow carried into the early Solar System. Clayton^{5,6} developed a picture treating most anomalies as extinct radioactivities trapped in mineral condensations in the expanding sites of explosive nucleosynthesis, presumably supernovae or novae⁷. As evidence for supernovae condensation grows, it becomes clear that one wants not only isotopic but

also mineralogical evidence of presolar grains. What supernovae condensates bearing unique signature are likely to survive? We advance arguments here that one should search among the ubiquitous sulphides present in meteorites, searching especially for sulphides of titanium. Our reasoning is that many sulphides, especially of titanium, will not be expected in solar condensation sequences but are expected to dominate certain key zones of supernovae expansion.

The element sulphur seems to have resulted primarily from the nuclear explosions of oxygen and silicon. Woosley *et al.*⁸ have analysed the arguments leading to that conclusion. The sulphur yield becomes more abundant than the oxygen fuel when about three-quarters of the oxygen has fused, and is more than 10^4 times more abundant when oxygen burning merges into silicon burning⁹. It is around this later time that the titanium abundance rises into equilibrium with those of Si, S and Ca. Therefore, if we concentrate on those supernova shells where Ti has been synthesised, we find $S/O > 10^4$. There is also no hydrogen. The elements Mg and Al are very deficient; roughly, $Mg/Si \approx 10^{-3}$ and $Al/Si \approx 10^{-3}$ while $S/Si \approx 1/2$. Shortly thereafter, the elements Cr, Fe and Ni rise to their natural abundance, without severely affecting key elemental abundances in the range $16 < A < 48$. Only Cl, K and the neutron-rich isotopes of S, Ar and Ca fall as the iron group rises. Here it is clear that following the few years required for that nuclear explosion to cool to 2000 K (refs 6, 10) the chemical condensation will proceed along unfamiliar lines. If this expanding supernova shell is not mixed by instabilities with oxygen-rich layers, the elements Si, Ca, Ti and, in sufficiently consumed Si burning, the iron group will condense in a sulphurous atmosphere. In those circumstances, on general chemical principles, sulphur is expected to play the role that oxygen normally plays in model solar condensation sequences¹¹. It will be of enormous interest to compute analogous supernova condensation sequences, but, because many of the necessary thermodynamic data on sulphide minerals do not seem to be readily available, the plausibility of titanium sulphides is here argued for the purposes of advancing this type of argument and of calling attention to needed mineralogical research.

In conventional solar condensation sequences¹¹, Ti condenses completely and early as a sequence of oxides: perovskite $CaTiO_3$ at 1647 K, Ti_3O_5 at 1393 K, and rutile TiO_2 at 1139 K, all computed at a total pressure of 10^{-3} atm. Similar sequences with small temperature decreases characterise lower nebula pressures. The Ti is locked up as oxides through the falling temperatures, and free Ti and sulphides of Ti do not exist in the equilibrium calculations. In the expansion from the silicon-burning shells of supernovae, on the other hand, the abundances of key elements stand in the approximate ratio $Si : S : Ca : Ti \approx 10 : 5 : 0.7 : 0.03$, so that an analogous sequence of sulphides will be expected. Since a complete set of thermodynamic properties of sulphides is currently sketchy, at present it is not possible to predict exactly such a chemical condensation sequence. Hence we confine ourselves merely to pointing out that sulphides are expected to condense. Among them, titanium sulphides are especially interesting because their chemical properties, such as insolubility in H_2O , HCl , HF and solubility in concentrated HNO_3 , closely resemble those of the puzzling mineral Q comprising less than 10^{-3} of the carbonaceous chondrite Allende¹². Q is presumed to be a double sulphide of Fe and Cr, which can also easily condense in the silicon-burning ejecta. In fact, in late silicon burning, when Fe/S becomes greater than unity, most of the S may condense as FeS , so that some of the common meteoritic troilite may also have presolar origin. We concentrate instead on the titanium sulphides in the belief that they will not be synthesised in the solar condensation sequence.

Metallic sulphides grains, as postulated above, will not alone be diagnostic of condensation in a supernova-shell, as these can be formed in carbon star atmospheres too. While detailed chemical thermodynamic calculations are required to reveal the mineralogical differences between the two grains, an isotopic

analysis may help distinguish between the two. For example, the most straightforward prediction from middle-to-late silicon burning is that presolar sulphides of Ti, Cr and Fe should be deficient in the two heaviest isotopes of S; that is, they should ideally be pure ^{32}S , although astrophysical instabilities may well mix the idealised layers. Early in silicon burning, however, when ^{33}S and ^{34}S are normal, a large fraction of Ti is concentrated into the light isotope ^{46}Ti ; therefore, presolar titanium sulphides rich in ^{46}Ti are also to be expected when accompanied by normal S. Similarly, if presolar calcium sulphides survive, one finds that $^{34}\text{S}/^{32}\text{S}$ correlates with $^{42}\text{Ca}/^{40}\text{Ca}$, being normal early in the burning and falling to zero as the iron group rises. So our immediate suggestion is that the ubiquitous strange sulphides, especially containing Ti, are good candidates for isotopic analysis.

In suggesting that strange sulphide minerals may provide mineralogical evidence of presolar grains, we are assuming that solar condensation does not yield the same minerals. In the case of titanium, it seems likely that the refractory oxides made in the solar sequence will not be reduced later by H_2S , in which case it seems that no titanium sulphides are possible in the usual picture. Lord¹³ has made this conclusion, but it may warrant a modern attack if the sulphides can be found. At present we offer this discussion in the belief that it could lead to important discoveries for the sciences of nucleosynthesis and the origin of the Solar System.

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The first metamorphic sodic amphibole identified from the Newfoundland Appalachians—its occurrence, composition and possible tectonic implications

MODELS for the emplacement of the western Newfoundland ophiolite suites include obduction over an eastward-dipping subduction zone¹, but no blueschist facies assemblages, considered to form in response to the high P/T gradients associated with subduction, have yet been identified in this area. Magnesioriebeckite and crossite which occur in meta-volcanics from the vicinity of Croque, northwestern Newfoundland, provide the first indication that these conditions may have been approached. This report deals with the petrographic relations, composition and probable conditions of formation of the Newfoundland sodic amphiboles, the latest in a series of occurrences described from Vermont^{2,3}, Quebec⁴ and New Brunswick^{5,6} (Fig. 1).

The host rocks are undeformed metabasalts which occur at the structural base of the Maiden Point Formation, at

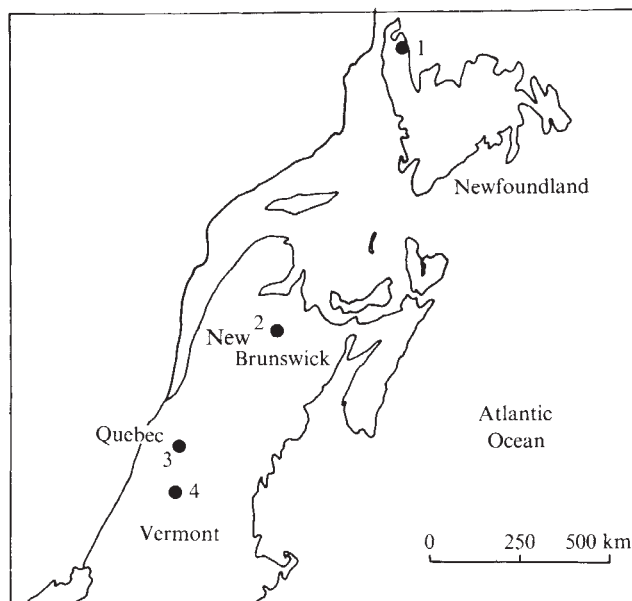


Fig. 1 A geographical location map showing the occurrence of sodic amphibole in the northern Appalachians. 1, Maiden Point Formation volcanics, Croque, Newfoundland (this paper); 2, Tetagouche Group Volcanics, New Brunswick^{5,6}; 3, Tibbet Hill Volcanics, Quebec⁴; 4, Hazen's Notch Formation, Vermont^{2,3}.

the bottom of the Hare Bay Allochthon, a sequence of sedimentary, metamorphic and igneous rocks which includes a partial ophiolite at its top⁷. The greenschist facies mineral assemblage albite–epidote–actinolite–chlorite–sphene–stilpnomelane–magnetite–quartz–(white mica) replaces the original pyroxene and plagioclase. There is little or no preferred orientation of the minerals, and the outlines of the pre-existing plagioclase phenocrysts are often preserved. Actinolite and brown stilpnomelane appear to post-date all other phases, growing as well developed

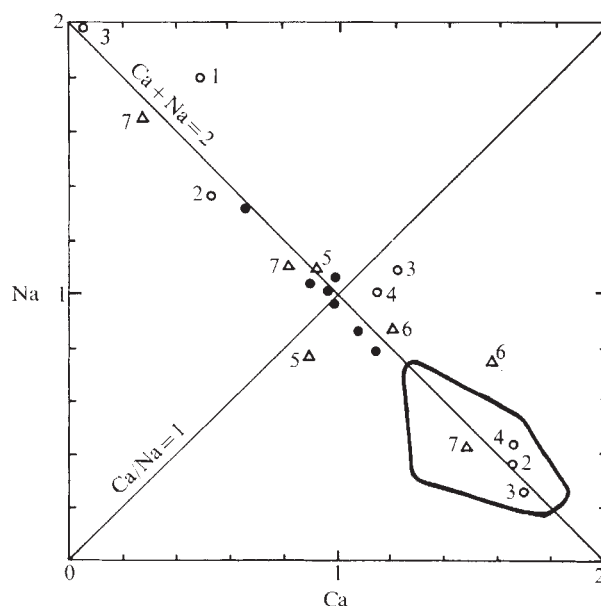


Fig. 2 The compositional range in terms of sodium and calcium content of amphiboles from the Croque metavolcanics (●). The outlined field includes all actinolite compositions from the area. Also plotted are amphibole compositions from elsewhere in the Appalachians (○) and other intermediate sodic–calcic compositions (△). 1, New Brunswick (H. Helmstaedt, private communication); 2, Quebec⁴; 3, Vermont (representative analyses of end members from the Tillotson Peak area, (J. Laird, private communication); 4, Vermont³; 5, Labrador¹³; 6, Switzerland¹⁰; 7, Washington¹¹.

acicular or sheaflike grains across a fine-grained mat of chlorite, epidote, albite and sphene. Actinolite and stilpnomelane do not appear in the matrix. Rare veinlets of albite, epidote and quartz transect the groundmass.

Amphibole with the pleochroic scheme X=yellow-green, Y=blue-violet, Z=indigo occurs in three samples from a suite originally collected by Smyth⁷. It rarely forms discrete grains, generally occurring as irregular zones or patches within actinolite crystals. Where a regular relationship can be defined, the blue amphibole tends to occur as cores rimmed by actinolite. No sharp boundaries between the actinolite and the sodic amphibole grains are visible. Where both amphiboles are present, actinolite is always the more abundant phase.

Table 1 Representative analyses of amphiboles from the Croque metavolcanics

	Magnesio- riebeckite RS 78-69/4-4	Intermediate sodic-calcic amphibole RS 71-172/1-3	Actinolite RS 165-70/2-3
SiO ₂	52.59	51.57	54.95
TiO ₂	0.06	0.05	0.02
Al ₂ O ₃	2.57	4.85	1.16
Fe ₂ O ₃ *	7.69	6.66	2.66
FeO	15.52	11.67	11.56
MnO	0.30	0.59	0.31
MgO	9.18	12.17	14.96
CaO	4.09	7.32	9.89
Na ₂ O	4.57	2.82	1.63
K ₂ O	0.05	0.07	0.05
Total	96.62	97.77	97.19
Structural formula on the basis of 23 oxygens			
Si	7.84	7.49	7.93
Al ^{iv}	0.16	0.51	0.07
Al ^{vi}	0.29	0.32	0.12
Ti	0.01	0.01	0.00
Fe ³⁺ *	0.86	0.73	0.29
Fe ²⁺	1.94	1.42	1.39
Mn	0.04	0.07	0.04
Mg	2.04	2.64	3.22
Ca	0.65	1.14	1.53
Na	1.32	0.79	0.46
K	0.01	0.01	0.01

*Ferric iron was estimated according to the method of Papike *et al.*²⁰

Microprobe analyses show that the amphiboles range continuously from sodic to calcic compositions (Table 1). The calcium-rich amphiboles have the low Al content and nearly ideal M4 site occupancy ($\text{Ca} + \text{Na} = 2.0$) that identify them as actinolites⁸ (Fig. 2). Approximately one-quarter of the analysed actinolites are unusually rich in sodium, with $1.2 < \text{Ca} < 1.5$ formula units, and are transitional into sodic compositions, with $\text{Ca} < 1.2$ units. The sodic amphiboles are best described as magnesioriebeckite (Fig. 3), with one analysed grain containing enough octahedral Al to be classified as crossite. Although the range of compositions between actinolite and magnesioriebeckite appears to be continuous when all the analyses are considered, each of the three samples studied displays a somewhat different set of compositions. RS 78-69 contains no analysed grains with $1.0 < \text{Ca} < 1.4$, RS 71-172 has a complete range between 1.0 and 1.8 Ca formula units, and RS 165-70 contains no amphibole with $\text{Ca} < 1.2$ units.

Studies of amphiboles from blueschist facies terrains have pointed to the existence of a miscibility gap between sodic and calcic compositions⁹⁻¹² and intermediate compositions, while rare, are not unknown (Fig. 2). Klein¹⁰ cites one case of coexisting hornblende and sodic actinolite from a metamorphic ophiolite in the Swiss Alps. Because of lack of evidence for equilibrium coexistence and the associated epidote-amphibolite facies mineralogy, this pair was thought to have formed at pressures and temperatures

above the proposed miscibility gap. Metamorphic conditions of $P > 6-10$ kbar and $T < 600$ °C were estimated for the formation of intermediate 'riebeckite-tremolite' from a metamorphosed iron formation in Labrador¹³. Amphiboles from the Shuksan region, Washington, show continuous zoning from crossite to actinolite¹⁴. In this case, Brown concluded that with increasing dT/dP the pair sodic amphibole—calcic amphibole is replaced by a single intermediate amphibole, which in turn is replaced by Na-rich actinolite and iron oxide. If this inferred relationship is correct and applicable to the Croque metavolcanics, sample RS 78-69 would have formed under conditions of relatively low dT/dP , since it is the only sample to show any gap between sodic and calcic compositions and also contains the most sodium-rich amphiboles. Samples RS 71-172, with its intermediate compositions, and RS 165-70, with its sodic actinolites, would have formed under progressively higher T/P gradients.

The presence of sodic amphibole alone is not sufficient to define a blueschist facies assemblage¹⁵, particularly in this case where it is an uncommon phase and has a magnesioriebeckite—crossite composition rather than one close to glaucophane. The stability of magnesioriebeckite ranges from authigenic to magmatic conditions⁸, and is probably very sensitive to bulk rock composition and f_{O_2} as well as pressure and temperature. The stability field of crossite has not been experimentally defined, but probably lies between those of glaucophane and magnesioriebeckite. The metamorphic conditions at Croque cannot be deduced from the presence of this phase alone, but must be related to the mineral assemblage as a whole.

Brown¹⁶ has suggested that the reactions chlorite + K-feldspar = stilpnomelane + actinolite and chlorite + K-feldspar + magnetite = muscovite + stilpnomelane + actinolite occur within the chlorite zone of the greenschist facies, at relatively high pressures and over a wide range of temperatures. The reaction curves, with negative slopes, lie at lower pressures on a schematic diagram than the stability fields of glaucophane and lawsonite, although they may intersect the blueschist facies curves at high pressures

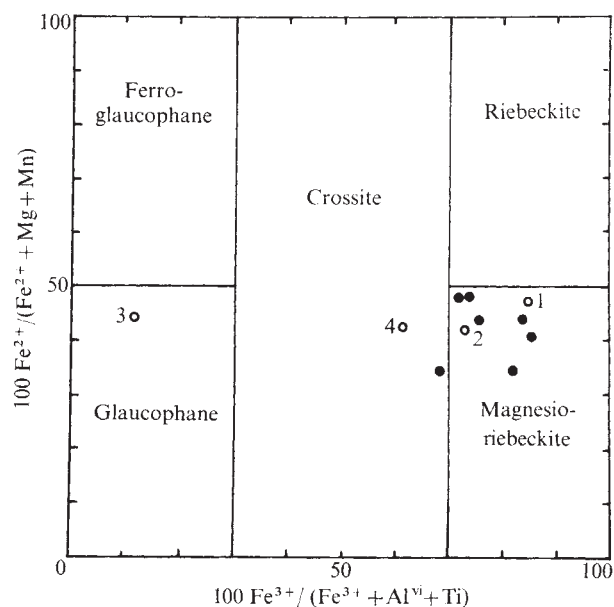


Fig. 3 Compositions of sodic amphiboles from Croque (●) in terms of glaucophane-riebeckite end members. Also plotted are amphibole compositions from elsewhere in the Appalachians (○). Ferric iron was estimated by the method of Papike *et al.*²⁰. 1, New Brunswick (H. Helmstaedt, personal communication); 2, Quebec⁴; 3, Vermont (J. Laird, personal communication); 4, Vermont³.

and very low temperatures. The mineral assemblages observed in the Croque metavolcanics seem to exemplify the proposed reaction relationships, with stilpnomelane + actinolite overgrowing the earlier chlorite-rich greenschist facies assemblage. White mica replaces feldspar in at least one example. On this basis it seems likely that the assemblages formed within the greenschist facies at rather high pressures, with one sample representing a possible transition to blueschist facies conditions.

Magnesioriebeckite occurs in comparable stratigraphic positions elsewhere in the Canadian Appalachians. Trzcinski⁴ speculated, on the basis of 'crossite' in Tibbet Hill Volcanics of Quebec, that blueschist facies metamorphism had occurred in association with a subduction zone. However, Fig. 3 shows that the sodic amphibole in question is magnesioriebeckite, similar to the Croque amphiboles in composition. The presence of chlorite, stilpnomelane, actinolite and white mica in the assemblage, together with the similar stratigraphic position in volcanics which underlie a Cambro-Ordovician flysch sequence strongly suggest that the metamorphism occurred under conditions very similar to those in western Newfoundland. Magnesioriebeckite has also been observed in similar assemblages in Lower Palaeozoic volcanics in the New Brunswick Appalachians^{5,6}. An amphibole with optical properties very similar to the sodic amphiboles in the Croque assemblage has been observed replacing chlorite in blocks of greywacke from the basal melange of the Humber Arm Allochthon in southwestern Newfoundland (R. K. Stevens, private communication). Glaucophane, barroisite and omphacite associated with Taconic metamorphism in Vermont^{2,3} suggest that a higher P/T gradient was achieved in that region.

The structural position of the Croque metavolcanics and the lack of a tectonic fabric are consistent with metamorphism resulting from deep burial. However, only 3.4 km of overlying material can presently be accounted for in the structural sequence¹⁷, although the mineral assemblages suggest that pressures of at least 3–4 kbar were involved. Current models for the onset of obduction in western Newfoundland would predict initial deep burial of the continental margin in response to loading by the overriding oceanic plate¹⁸. Later isostatic rebound and collapse of the continental margin sequence into the series of thrust sheets which is now observed would lead to the emplacement of the volcanics and associated greywackes into their present structural position. This model is supported by the presence of eclogitic dykes in the underlying, more deeply buried, Grenville basement to the east¹⁹. It would, however, represent conditions with lower P/T gradients than those reflected in the Franciscan 'subduction melange' of California and similar sequences where complete blueschist facies assemblages are common.

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Global climate change during the 13,500-b.p. Gothenburg geomagnetic excursion

ROBERTS and Olsen¹ suggested that high latitude atmospheric ionisation has an important role in nucleating cirrus clouds. And Harrison and Prospero² deduce that, if this is correct, severe drops of the Earth's magnetic field strength, such as are believed to accompany reversals, would lead to increased upper atmospheric cloudiness at all latitudes and correspondingly to major climatic events. There is likely to be warming at high latitudes but considerable cooling in the middle latitudes, in a way analogous to the aerosols³.

I decided to test this idea in connection with the Gothenburg Excursion, the youngest well-documented geomagnetic event in the geological record. Mörner and Lanser⁴ have shown this episode to have been short-lived, only 13,750–12,350 yr b.p., and Creer *et al.*⁵ on the cores from Lake Erie, but with less precise dating, identified the excursion only to within 14,000–11,000 yr b.p. The global nature of this magnetic disturbance is indicated by other dates from the Gulf of Mexico, Japan, France and Australia, and often correlated with the poorly dated 'Laschamp' Event.

What happened at that time to global climates? While no cause and effect relationship can be claimed at this stage, it is useful to point out that a number of dramatic events 'happened' at the same time, for example (Fig. 1):

North American glacial record. The great Laurentian ice sheet was in full retreat by 15,000 b.p. and was about one-third melted. But suddenly, according to Dreimanis and Goldthwait⁶ the glaciers ceased their retreat and reoccupied approximately 500,000 km² of southern Canada and the American mid-west. This readvance over a distance of 550 km (Carey, Port Stanley Till) took place about 14,000 b.p. with a last oscillation at 13,000–12,700 b.p. (Port Huron). A corresponding but smaller readvance is indicated in the north-European record⁷, and data from New Zealand and Patagonia also suggest a return of the glaciers.

Eustatic evidence of glacier volumes. Mörner⁸ gave the amplitude of the negative eustatic oscillation in the interval 13,000–14,000 b.p. as 9–12 m. Inasmuch as 1 mm of glacio-eustatic change represents 360 km³ of sea water, a 10 m oscillation would involve 3.6×10^6 km³ of water or 4×10^6 km³ of ice. If correct, these observations suggest that within a thousand-year interval a volume of ice was newly formed that was equivalent to 20 times all of the world's present mountain glaciers put together and a third more than all the ice in Greenland today.

Central African rainfall and Nile discharge. During the last glacial stage Lake Victoria (head of the White Nile) became a carbonate-rich evaporating lake and did not overflow. Precipitation was likewise very low in Ethiopia and the Blue Nile was severely impoverished. The middle valley of the Nile became the limit of flow and gradually became silted up with a vast wedge of sediments that built up to a height of 40 m above the present level of the Nile bed, according to my own surveys⁹. Comparable desiccations of other African rivers such as the Niger and Senegal are now established¹⁰. Even the Congo was locally overwhelmed by

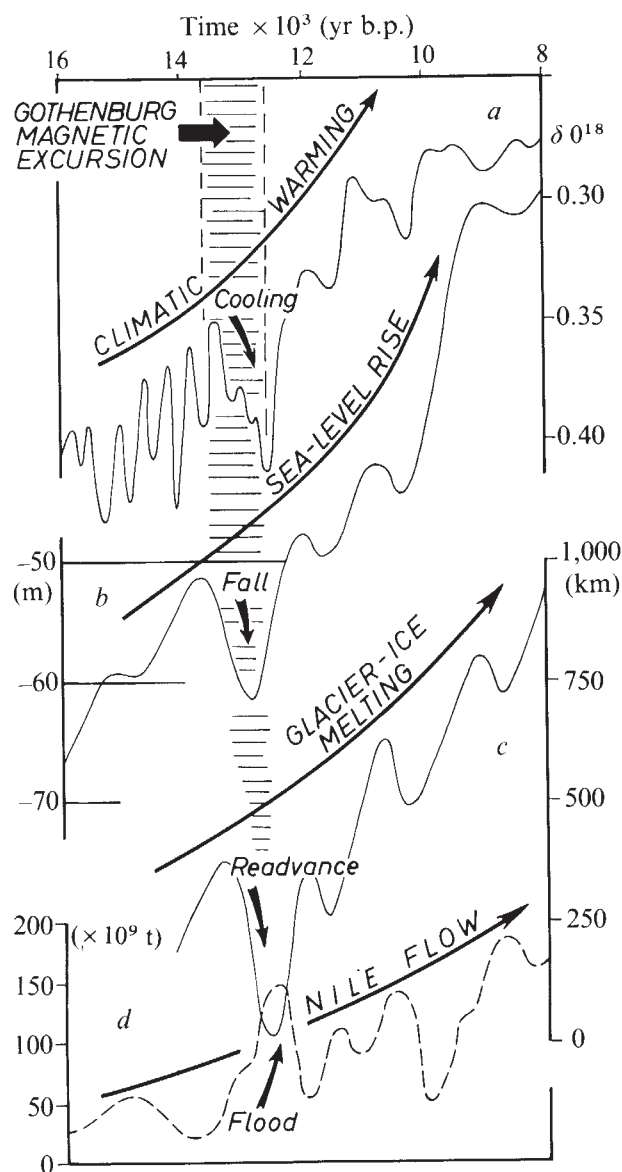


Fig. 1 Four climatic indicator curves for the period 16,000 to 8,000 b.p. The Gothenburg palaeomagnetic instability was approximately 13,750–12,350 b.p. according to Möner and Lanser⁴. a, Climatic warming reflected by $^{18}\text{O}/^{16}\text{O}$ ice records from Camp Century, Greenland; b, sea-level rise, from data assembled by me; c, glacier-ice melting, measured as km of retreat in southern Canada⁶; d, Nile discharge, measured from the sedimentary cut and fill levels in Nubia⁹.

dune sand¹¹. The silt wedge of the Nile was dramatically cut through around 13,500 b.p. by an enormously augmented flow (at least four times the present discharge) and after a series of oscillations the talweg reached bedrock in many areas and the present regime was established. Sedimentation in the eastern Mediterranean seaward of the Nile delta up till around this time was a white, pelagic ooze, but then became abruptly followed by fine, terrigenous silts introduced by the Nile, which speaks for a sudden change in the fluvial regime.

It is clear from the discoveries of the last 15 yr (ref. 12) that the Milankovitch planetary insolation pattern shows a convincing correlation with gross planetary climate changes within a 92, 40, 26×10^3 yr framework, and dynamic calculations seem to support the model¹³. Nevertheless, this theory offers us no hint of an explanation for brief climatic

fluctuations or excursions on the scale of decades up to 5×10^3 yr.

The evidence of the Gothenburg fluctuation suggests that a major change in the normal global heat transfer was involved that seems to call for a non-steady state explanation. As recently recalculated by Berger¹⁴, there was also a critical seasonality change affecting the 'sensitive' latitudes at about the same time as the Gothenburg excursion. This time was roughly about the mid-point in the gigantic melting that followed the last glacial maximum. It is difficult to relate it in any way to that warming trend. Quite the contrary, it correlates with a very brief but intense cooling. During the great melting, which lasted 10,000 yr, the sea level rose 135 m¹⁵ and considerably changed the geography of land and water. Could a negative feedback have caused the Gothenburg cooling? In general the changes of albedo on a warming Earth combined with increasing dimensions of oceanic areas would lead to positive feedback, thus accelerating the melting. Indeed, the resultant exchange of mass loading on the Earth may very well be the cause of the magnetic instability itself¹⁶. The increased pluviosity of the equatorial regions at this time suggested by the Nile and other fluvial regimes might indicate a sudden drop in the continental evaporation rate.

Another curious coincidence seems to match the timing of the Gothenburg Excursion. Kopper¹⁷ has demonstrated that in the evolution of man, each of the key subspecies introductions or cultural boundary horizons seems to correspond with an important magnetic reversal or excursion. The Gothenburg event appears to mark the end of the Neanderthals.

Most recently another report of this magnetic excursion was obtained from Blekinge, south-east Sweden, by Noel¹⁸, but dated by uncorrected varve chronology as 12,077–12,103 $\pm$ 150 b.p. This controversial dating may be up to 300 years too young.

In summary, it should be emphasised that this proposed magnetic-climatic relationship is strictly limited to events on a scale of up to 10^3 yr and is in no way a substitute for the Milankovitch modulation of ice-age climates, although in certain instances it may play a triggering role.

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Dry years in south-east England since 1698

THE recent drought and water shortage in England has re-emphasized the need for a more detailed knowledge and better understanding of the climate of the past. Without such information it is impossible to make meaningful statistical

Table 1 Years of greatest soil moisture deficit for south-east England, 1871–1970

Rank	1	2	3	4	5	6	7	8	9	10	11
From daily data	1934	1893	1921	1938	1943	1959	1896	1961	1929	1944	1965
From monthly data	1934	1944	1893	1938	1921	1901	1965	1896	1949	1929	1943

statements about the future occurrence of such extreme events. Many meteorological statements have been made about the severity and causes of the 1976 drought, mainly in the popular press, but also in the scientific literature¹. These statements have generally concerned precipitation; but data on precipitation alone are not necessarily good indicators of drought. In this paper we distinguish between three different kinds of drought—meteorological, hydrological and agricultural drought—noting that agricultural and hydrological drought conditions are determined, not only by rainfall, but also by evaporation and by the timing of rainfall events. Using both precipitation and evaporation data for Kew we derive a time series of soil moisture deficits which we believe gives a more rational measure of agricultural drought severity than can be obtained from precipitation data alone.

Drought conditions are not determined solely by lack of rainfall. The precise evaluation of drought severity is difficult because different people perceive drought in different ways. To many people a 'drought' is a long period with little or no rainfall; indeed this is the basis of the definition introduced by Symons². A more useful impression of drought severity is given by precipitation deficiency diagrams in which accumulated precipitation is compared with the long-term average for the station³. Accumulated departures from the average are a measure of drought severity. We may refer to a drought defined solely by deficiency of precipitation as a 'meteorological drought'. Though interesting in its own right, this is not a particularly useful definition of drought and Rodda⁴ has stated unequivocally that "... a drought definition based solely on the absence of rain is hydrologically unrealistic". To quote Rodda further: "... though the causes of drought are meteorological its definition is best made in hydrological terms".

To industry and consumers of water, a drought occurs

when water supplies become low. Thus a second type of drought, 'hydrological drought', is the result of a deficiency in 'excess' rainfall over some preceding period. 'Excess' precipitation is that which is neither evaporated nor used up in restoring soil moisture deficits and is therefore available to form surface run off and/or groundwater recharge. The length of a hydrological drought depends on the size of the region and the type of water resource affected⁵.

To the farmer, drought conditions are those which lead to poor crop yields. An 'agricultural drought' will therefore be determined largely by conditions which lead to stunted growth or even wilting of crop plants. These are a combination of dry soil and a continuing high level of potential evapotranspiration, which the crop is unable to maintain by withdrawing water from the soil. This combination leads to a consistently high soil moisture deficit over the growing season. Thus the average soil moisture deficit over the 4-month period May to August can be used as an index of drought severity. (It is worth noting in passing the the opposite condition, of very wet soil during the growing season is equally adverse for crop yields.)

Definitions of drought have been exhaustively reviewed by Hounam *et al.*⁶ who also point out the distinctions between different types of drought. Rodda *et al.*⁵ in their discussion of drought indices conclude that soil moisture deficits provide the best practical drought index. Soil moisture deficits are the result of losses of soil moisture in periods when evapotranspiration exceeds rainfall. These losses depend on the type of vegetative cover; once the soil moisture is depleted below an effective root level, evapotranspiration can only proceed very slowly, at a rate much below the potential evapotranspiration rate which would occur from a fully wet soil. To account for the influence of vegetation type, and for the differences be-

Table 2 Growing-season mean soil moisture deficits for Kew, 1698–1976

	0	1	2	3	4	5	6	7	8	9
1690									29.4	100.5
1700	109.9	93.1	66.4	13.5	103.8	113.9	64.8	107.5	68.0	51.3
1710	80.9	64.6	61.9	29.4	100.0	51.6	95.3	72.0	80.8	92.9
1720	47.8	59.1	68.9	109.8	95.7	67.4	75.6	68.5	22.6	85.3
1730	78.3	114.8	76.7	86.8	37.7	60.2	95.6	89.4	76.1	64.1
1740	86.4	86.7	98.6	108.0	105.9	57.6	76.5	98.4	86.7	97.5
1750	96.6	53.9	85.0	100.4	94.9	67.1	71.8	89.2	73.2	93.9
1760	110.8	99.2	113.7	91.9	85.8	90.9	17.7	20.3	31.6	60.6
1770	45.8	79.8	90.7	60.0	77.2	94.9	93.4	11.2	100.8	69.1
1780	98.7	115.5	16.6	75.9	63.4	108.5	97.9	82.6	100.6	43.9
1790	67.9	98.7	58.3	97.9	94.0	83.7	101.7	58.1	97.4	71.3
1800	88.7	82.6	92.1	86.2	81.0	34.0	81.9	100.0	89.1	79.6
1810	80.7	67.8	34.2	84.1	59.9	93.5	6.6	56.3	94.0	63.7
1820	72.0	45.5	88.4	75.8	21.2	73.1	92.1	92.8	59.6	46.8
1830	54.1	67.7	69.7	87.0	73.8	72.2	81.4	88.3	101.8	66.3
1840	105.1	67.6	101.7	50.3	114.9	70.8	90.2	108.4	74.4	63.0
1850	95.7	80.4	91.1	47.9	78.3	83.7	63.8	99.6	80.0	97.9
1860	9.9	94.4	36.2	99.1	106.6	97.6	69.8	83.8	111.5	81.5
1870	112.7	76.8	67.0	90.5	111.7	75.4	93.3	64.8	12.8	0.0
1880	85.5	97.0	82.2	86.1	97.3	85.2	62.1	96.6	46.6	43.5
1890	36.7	65.9	89.7	118.1	61.8	104.9	113.3	95.3	105.9	94.6
1900	102.4	115.2	82.4	0.1	85.3	91.3	98.6	58.5	79.8	83.0
1910	77.1	92.8	79.2	84.3	90.4	64.9	74.9	27.3	69.0	96.8
1920	58.4	116.5	106.4	81.7	22.9	86.0	61.2	71.5	78.9	112.5
1930	58.3	37.4	66.0	97.7	123.6	82.9	81.4	65.8	117.3	83.9
1940	99.8	41.1	92.7	112.0	121.9	87.7	24.5	86.7	111.0	112.7
1950	77.6	76.2	88.4	78.2	57.4	86.4	88.8	111.9	54.9	108.6
1960	108.4	108.4	99.6	99.8	62.9	114.0	63.8	53.9	51.9	86.8
1970	106.8	38.0	105.3	110.1	116.1	93.3	126.4			

tween actual and potential evapotranspiration, Penman⁷ introduced the concept of a root constant. Penman's original work indicated that potential and actual evapotranspiration rates were effectively equal for soil moisture deficits less than $C + 25$ mm (where C is the root constant (unit mm) a function of vegetation type). For greater soil moisture deficits actual evapotranspiration rates are considered to drop rapidly to about 10% of the potential rate at a soil moisture deficit of around $C + 37.5$ mm. Beyond this the ratio of actual to potential evapotranspiration rate is assumed to remain at about 10%. Although there are differences due to soil type, this simple model gives remarkably good results in water balance calculations over a wide range of conditions⁸.

Long homogeneous series of precipitation and evapotranspiration data for Kew going back to 1698 have been constructed and discussed by Wales-Smith⁹⁻¹¹; these are the longest such series available anywhere in the world. From these data one can determine, month by month, the soil moisture deficit (D) for different types of vegetative cover. D values are generally calculated by using the method of Penman⁷. The Ministry of Agriculture and the Meteorological Office publish D values for different regions at regular intervals, and the Meteorological Office has calculated D values for Kew back to 1871 using the Penman approach as discussed by Grindley⁸.

We have reconstructed monthly D values back to 1698 using the same approach and the Kew data. For most of this period only monthly data are available so it has been necessary, in order to obtain a homogeneous series, to use monthly data throughout. It is, however, preferable to use daily precipitation and evaporation data. Grindley⁸ has pointed out that, because precipitation is a sporadic process, the use of monthly data alone can produce significant errors in estimating month-end D . We have found that these errors can be reduced by dividing each month into ten segments, distributing monthly precipitation and potential evapotranspiration values evenly over these segments, and then calculating soil moisture deficits accumulated

this value is too high⁵.) The results are representative of south-east England: the Kew precipitation record correlates extremely well ($r = 0.89$) with the south-eastern England precipitation series constructed by Gray¹² for the period 1840–1970.

To test our method of soil moisture deficit calculation from monthly data we have compared our results with those calculated by the Meteorological Office using daily data. The Meteorological Office series gives the mean D over a standard catchment containing 50% short-rooted vegetation with a root constant of 75 mm, 30% long-rooted vegetation with a root constant of 200 mm, and 20% riparian land where a soil moisture deficit is never established. Using this standard catchment, the correlation between D values calculated from daily and from monthly data is extremely high; approximately 0.95 for both the 1200 month-end values and the 100 growing season (May to August) mean D values for the period 1871–1970. Furthermore, there is excellent agreement between the years with extreme D values (based on growing-season mean D) calculated using both methods. Table 1 shows the 11 'worst' years over the 100-yr test period ranked in order of decreasing mean D .

The similarity between these rankings is remarkably good when one considers that the 6th to 11th places cover a range of only 3.2 mm in mean D . The precise ranking is determined by very small differences in D . Furthermore, our results are in excellent agreement with those of Rodda⁴. His results for the period 1916–1964 show the most severe droughts as occurring in 1921, 1934, 1943 and 1944. Over the same period our data show 1921, 1934, 1938 and 1944 to be the most severe years. Rodda's results are based on data from Oxford while ours are based on data from Kew.

We have chosen to average D over the whole growing season, and to use a composite of short- and long-rooted vegetation results in order to provide a general agricultural drought index. Our index is thus a rather coarse one, and a high value will not always be an indicator of severe drought conditions for all plants. This makes a direct comparison with specific crop yields difficult, a problem which is accentuated by the influence that developments in agricultural technology have had on crop yields over the past century, which no purely environmental drought index can incorporate. Calculated growing season mean D (henceforth, D_m) values are given in Table 2.

Using this index, the 14 most severe agricultural droughts over the interval 1698–1976 are: 1705, 1731, 1762, 1781, 1844, 1893, 1901, 1921, 1934, 1938, 1944, 1965, 1974, 1976. The seven worst years are, in order, 1976, 1934, 1944, 1893, 1938, 1921 and 1974; all but one having occurred this century. 1976 shows up as the worst agricultural drought since at least 1698, although only fractionally more severe than 1934. Considering the method of constructing the series, however, it would be more realistic to say only that 1976 was among the three worst agricultural droughts during the 279-yr period of record. All three of these 'worst' droughts have occurred in the last 43 yr.

This is a strong indication that the series of D_m extremes is non-stationary. We tested this in the following manner. The series from 1707 to 1976 was divided into 27 10-yr periods and extreme D_m values for each decade were extracted and ranked. Recurrence intervals estimated by using the Gringorton¹³ formula were then plotted against $(D_m)^n$ on a semi-logarithmic plot for various n . A linear fit was only possible for large n ; $n = 12$ providing a good fit. From the linear fit the recurrence interval for the third highest value of D_m (1944) was estimated as 13.2 decades. In the last five decades (1927–1976) of the record, three events have occurred with equal or greater D_m (1934, 1944 and 1976). The probability of such a clustering occurring by chance, given a stationary series, is less than 0.4%. The null hypothesis that the series of decadal extremes is stationary must therefore be rejected.

An alternative estimate of the recurrence interval can be obtained by using the formula $(n+1)/m$ where n is the number of years in the record and m is the rank of a particular year⁴.

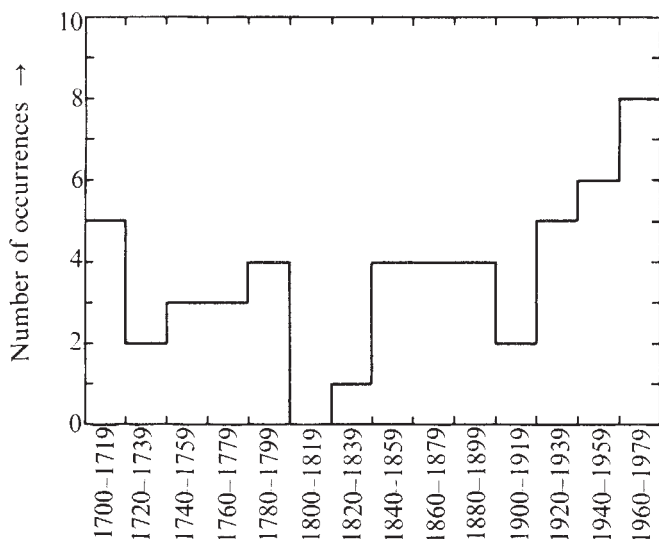


Fig. 1 Number of times the soil moisture deficit averaged over the growing season (average of month-end values for May, June, July and August) exceeded 100 mm in 20-yr intervals. The interval 1960–1979, which is still incomplete, has the highest number (8).

over successive segments. Using this procedure we have generated monthly values of D for both short ($C = 75$ mm) and long-rooted ($C = 200$ mm) vegetation for Kew from 1698 to 1976. (75 mm has commonly been used as a root constant for short-rooted vegetation although some authors have suggested that

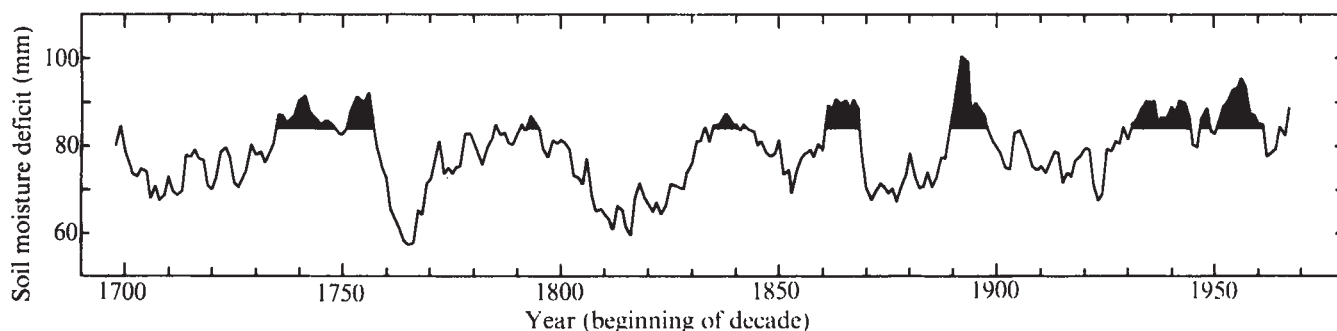


Fig. 2 Ten-yr running means of soil moisture deficit averaged over the growing season. The value of year n corresponds to the decade n to $n+9$ inclusive. An arbitrary datum level of 84 mm has been shown to accentuate the periods of higher deficits.

This gives a recurrence interval of 93.3 yr for the third highest D_m . Given a stationary series, the probability of three such events occurring in the past 50 yr is about 1.5%; a result essentially in accord with the previous analysis. The non-stationary of the extremes of the growing-season mean soil moisture deficit time series makes it extremely difficult to estimate the true return period for severe droughts.

Non-stationarity is further exemplified by the increased frequency of high D_m values this century: of the 14 worst years, 8 have occurred since 1900. This trend is illustrated in Fig. 1 which shows the number of times the growing-season mean D exceeded 100 mm in 20-yr intervals. The most noticeable features of Fig. 1 are the almost drought-free period from 1800 to 1839, and the fact that more high D_m years have occurred in the 17-yr period 1960–1976 than in any preceding 20-yr period. The drier soil conditions of this century are most probably associated with the warmer English climate which has prevailed since about 1880, although no effect of the cooling trend since 1940 is evident. This may indicate that urbanisation has had an effect on evaporation at Kew. Although we do not think such an effect can be large, it is a factor which warrants further investigation.

Another indication of the trend towards more frequent agricultural drought over the past 50 yr is given by Fig. 2 which shows 10-yr running means of average growing season D values. Figures 2 and 1 are not equivalent since the latter only isolates the extreme events. There are, however, some interesting comparisons. The recent trend to more frequent large D_m values does not show up in Fig. 2, although the period from 1930 to the present is the longest period of consistently high growing season soil moisture deficits since 1698, marginally 'worse' than that of the mid-eighteenth century.

We noted earlier that rainfall data alone do not provide a good indicator of agricultural drought. In support of this contention we found that D_m does not correlate particularly well with annual precipitation. The correlation with summer rainfall ($r = 0.80$) is somewhat better. However, in spite of this high correlation, the years of highest D_m do not in general correspond with the driest summers. This is because in addition to having low summer precipitation it is necessary to have established a high soil moisture deficit prior to the growing season, and to maintain high evapotranspiration rates during the growing season. Nevertheless, a good prediction of summer rainfall is a prerequisite for the prediction of agricultural drought: reliable rainfall estimates two to three months in advance appear to be essential.

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Structural relationships between corn yield and weather

REPORTS of crop-weather relationships are sparse in the literature and Fisher's classic paper in 1924 has not been improved upon¹. We find this surprising in view of current concern regarding the ability of the world to feed its rapidly increasing population. This paper reports on progress in our research dealing with corn-weather relationships, a subject of general interest as corn is grown over large areas of the globe.

The data are taken from selected parts of the main corn growing region in South Africa (Fig. 1), namely, four spatially representative sub-regions in the Orange Free State. The data, for the period 1949/50 to 1964/5 consist of corn yields in 200 lb bags per morgen, monthly rainfall totals and mean temperatures at 0800 and 1400; the rainfall and temperature data apply to months over the growing season. By using the method of principal components, these data are shown to be uncorrelated². Multiple regression analysis is applied to the data³. Yield is regressed in turn on rainfall, and temperature. The degrees of freedom dwindle, but this does not detract from the value of the strategy adopted. Time was also included in the regressions to account for any temporally varying yield stimuli not otherwise considered, such as fertiliser application. The importance of each month's rainfall and temperature in accounting for the

total sum of squares in yield is shown in Figs 2 and 3. Temperature data are presented for the recording time that contributed most strongly to the sum of squares for yield; the order of inclusion of a month into the regression is indicated by the number at the top of each rectangle.

All four districts are in the summer rainfall region of the country. The yields for Bethlehem and Kroonstad show least dependence on individual monthly rainfall totals (Fig. 2). This is probably because the mean annual rainfall for these two is somewhat greater than for Bloemfontein and Hoopstad, therefore, rainfall is less inhibiting to yield at Bethlehem and Kroonstad than at the former two locations. Bloemfontein is situated furthest south and is the only region where yield is sensitive to mid-season rainfall.

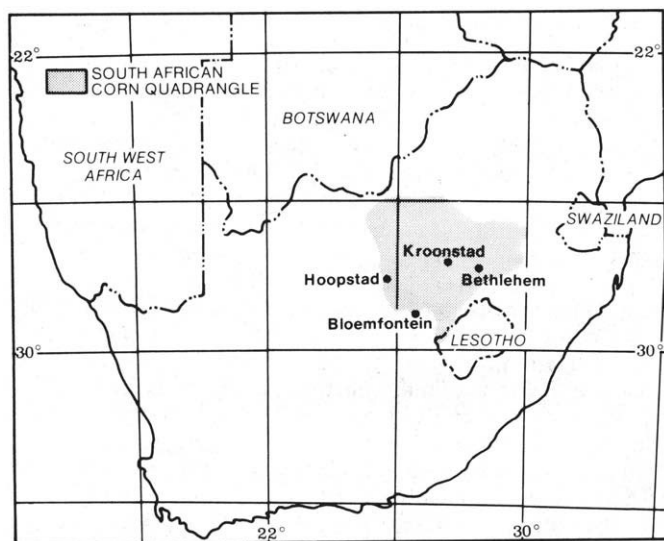


Fig. 1 The position of the South African main corn growing region (shaded), and the location of the sites considered in this paper.

The yield for Bethlehem is related to both early and late rains. In the case of Hoopstad, and to a lesser extent Kroonstad, it is the late rains which are critical to yield variation.

The partial correlation coefficients between yield and early rains are, significantly, negative. This applies to September for Bethlehem, as well as the early months for Kroonstad and Hoopstad. The late rains, and mid-season for Bloemfontein, have positive partial correlations with yield. The adverse effects of early rains result from intense rainfall coupled with instantaneous run-off wastage.

Fig. 2 The percentage of total sum of squares for corn yield accounted for by rain over the growing season.

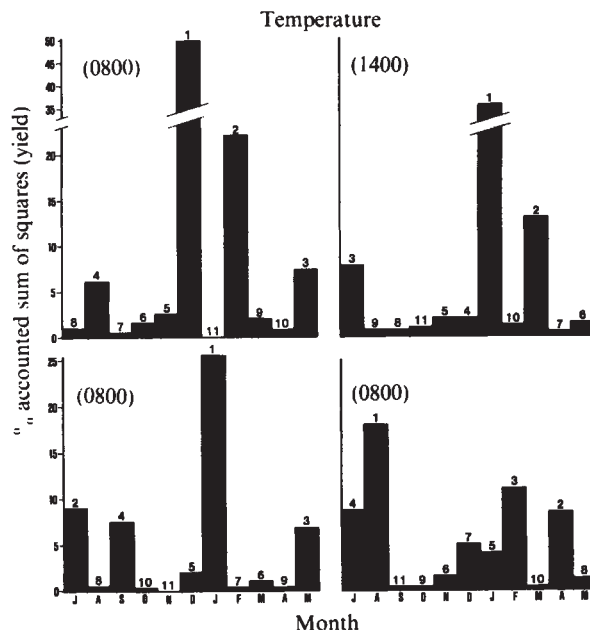
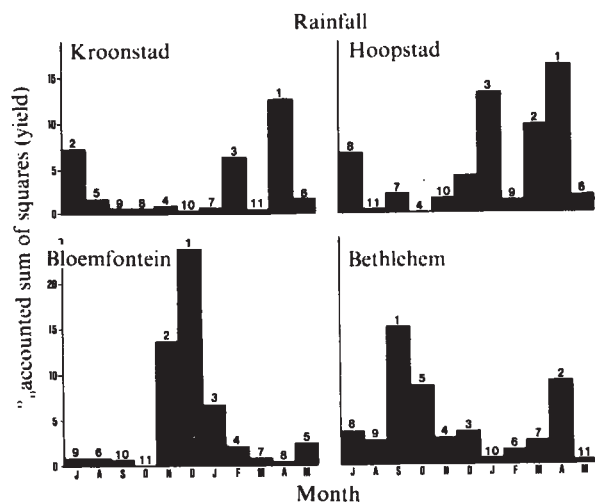


Fig. 3 The percentage of total sum of squares for corn yield accounted for by temperature over the growing season.

Yields at Bethlehem are least sensitive to temperature and when considered also in relation to rainfall, this is the most equitable region for corn. The yield has positive partial correlation with August's temperature. The mid-season temperature is important to yields in the other three regions. This applies particularly to Kroonstad which was little affected by rainfall. For this region the temperatures during December and February have negative partial correlations with corn yield while the converse is true for temperatures at the latter end of a season. This conformation is not unexpected; warm temperatures are required to assist germination, but warm temperatures concluding a season would result in loss of moisture from the crop, with consequent decreases in yield.

The most influential rainfall and temperature months were combined, and the regressions repeated. The structural relationships between yield, rainfall and temperature resulted in the following variables being drawn into the regressions. (1) Kroonstad: time, January, July, May, September and December temperatures. (2) Hoopstad: January temperature, time, March and July temperatures. (3) Bloemfontein: time, January, July, May, September and December temperatures. (4) Bethlehem: time, August temperature and September rainfall.

The percentage variance in yield accounted for by each regression is 85, 86, 95 and 70%, respectively, for factors 1, 2, 3 and 4 above. Note that, apart from time, temperature is the dominant limiting factor for yield. This emphasises the erroneous conclusions that may be drawn from an inspection of the simple correlation matrix. The advantages in using stepwise as an alternative to straightforward multiple regression become obvious. Arithmetic details relating to the analyses may be obtained from the authors.

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Methylated arsenic from marine fauna

It has long been known that certain species of marine animals contain arsenic in their tissues at concentrations up to 100 p.p.m. (ref. 1), and there is little doubt that this arsenic is naturally acquired and does not reflect environmental pollution. The toxicity of arsenic is dependent on its chemical environment and valency state^{2,3}. Since the demonstration of the rapid and complete excretion by the human kidney of arsenic consumed in fish and shellfish^{1,4}, it has been accepted that such arsenic is in an organic and nontoxic form⁵. Clearly full toxicological evaluation of the arsenic in marine animals awaits the elucidation of the precise chemical nature of that arsenic. We have found that mussels (*Mytilus edulis planulatus* Lamarck), western rock lobster (*Panulirus longipes cygnus* George), and stingray (*Dasyatis thetidis* Waite) contain dimethylated and trimethylated arsenic together with a small proportion of inorganic arsenic.

Lobsters were collected off the western shore of Garden Island and mussels and stingray were taken from Cockburn Sound, both in Western Australia. Total soft tissue from the mussels (containing 1 p.p.m. arsenic), tail muscle from the lobster (20 p.p.m. arsenic) and muscle from the disk of the stingray (20 p.p.m. arsenic) were used. Concentrations of arsenic were determined by vapour generation atomic absorption spectrophotometry⁶ after digestion of the samples in a mixture of nitric and perchloric acids. Digestion of methanol extracts of the above tissues in aqueous sodium hydroxide led, after neutralisation, to a solution from which arsines were generated on addition of sodium borohydride. Fractional distillation of these arsines from a cold trap into an atomic absorption spectrophotometer⁷ led to their identification and has provided information on the alkylation pattern of arsenic contained in fish and shellfish. Sodium borohydride treatment of the methanol extracts before sodium hydroxide digestion produced no volatile arsines.

Preliminary methanol extraction was carried out on macerated tissue of each species. Virtually all arsenic was removed from rock lobster and stingray tissues in this way; 60% of that in the mussel was recovered. The extracts were each treated as follows. Methanol was removed and the residue was digested in sodium hydroxide solution. A small portion of the digest was treated, after neutralisation and addition of buffer, pH 4–5, with aqueous sodium borohydride. The arsines generated were trapped in a tube packed with glass beads at the temperature of liquid nitrogen. The trap was linked to an atomic absorption spectrophotometer and allowed to warm, with periodic injection of the volatilised contents of the trap into the flame, as described before⁷. Comparison of the time taken for the arsines from lobster muscle digest to volatilise and enter the flame with those for the standards—arsine, methylarsine and dimethylarsine—revealed 5% unsubstituted arsine, 60% dimethylarsine and 35% of an arsine subsequently identified as trimethylarsine. The percentages represent the constituents of the neutralised base digest when the concentration of sodium hydroxide varied from 10 to 40%: the stronger the base, the greater the release of total arsenic susceptible to reduction. Sodium hydroxide in excess of 40% did not increase the total arsenic released. Digestion was always for 15 min.

Trimethylarsine was identified by use of rock lobster tissue only. The arsines produced by borohydride reduction of the sodium hydroxide digest were trapped by passing all effluent gases through toluene. After concentration, the toluene solution was subjected to gas chromatography and the peak corresponding to the unidentified arsine was located by smell at a sniffing port (strong garlic-like odour).

Trimethylarsine was identified by gas chromatography–mass spectrometry⁸.

In the sodium hydroxide digest inorganic arsenic is probably present as sodium arsenate or arsenite and dimethylated arsenic as sodium dimethylarsinate. The form of the trimethylated arsenic is more doubtful. Trimethylarsine oxide seems the most likely. The possibility that trimethylated arsenic was demethylating in the alkaline digestion conditions was discounted because the proportions of di- and trimethylated arsenic did not change within the range of sodium hydroxide concentrations that brought about their release. Also, the resistance of dimethyl (trifluoromethyl) arsine to hydrolysis⁹ indicated that demethylation would not occur in even the strongest basic conditions used. The close similarities in proportions of the various arsenic compounds from the different species examined may indicate the partial breakdown, in the alkaline conditions used, of a single complex arsenical. That such dissimilar species should all contain arsenic in a single form may seem more likely than that all three should contain the same proportions of three arsenicals. There are, however, no observations on which to base such speculation. Work is in progress to elucidate the chemical structures associated with the methylated arsenicals in both the methanol extract and the sodium hydroxide digest. Preliminary work on the urine of subjects consuming fish containing arsenic suggests that the arsenic remains in a bound state in the body and is excreted in that form. Work is being continued on this aspect of the study.

Although we are still some way from the stage where detailed toxicological assessment of arsenic in marine fauna can be made, it is evident that most arsenic in such situations is organically bound and the general acceptance of the nontoxic nature of this arsenic on such grounds seems to be justified.

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Jurassic labyrinthodont

THE discovery of a member of the Labyrinthodontia in the Surat Basin of Queensland, Australia, brings the last known occurrence of this amphibian subclass firmly into the Jurassic.

The specimen was discovered on a property, Kolane, approximately 35 miles ENE of Taroom, within the oolitic member of the upper Evergreen Formation. As marine fossils are largely absent from Jurassic sediments of eastern Australia, the latter have been dated almost entirely by correlation of their microfloras with those of the Jurassic sequence in Western Australia the age of which has been determined by means of its marine faunas. Thus, the first appearance of *Tsugaepollenites segmentatus* and *T. dampieri* in the upper Evergreen clearly dates this formation

as Upper Liassic^{1,2}. In addition the possibility of an Upper Triassic age for the lower Evergreen Formation and the underlying precipice Sandstone is ruled out by the presence of *Classopolis classoides* which is not known from accurately dated pre-Jurassic sediments¹.

As far as can be ascertained before preparation, very little of the skeleton of the Kolane amphibian is missing so that, when prepared, this late survivor of the labyrinthodonts should also be one of the best known.

Many factors suggest that the specimen is a labyrinthodont amphibian. The teeth are labyrinthine in cross-section, the skull and dermal pectoral girdle are ornamented, and there are indications of sensory canal grooves on the skull. In addition, the shape of such limb or girdle bones which can be seen in cross-section is characteristic of Triassic members of the order Temnospondyli, as is the structure of the vertebrae with the neural arch detached from an enlarged intercentrum and a small unossified pleurocentrum.

The specimen is preserved in massive ironstone blocks, and preparation will present difficulties. But enough of the skull is exposed to allow determination down to familial level. The Kolane amphibian conforms to some of the most important criteria used by Welles and Estes³ and Cosgriff⁴ for inclusion of a skull in the family Brachyopidae. Its affinities within that group must remain uncertain pending its further preparation. The skull table is short and broad and on the right of the specimen the characteristic vertical plate of the pterygoid is exposed, while median to this the occipital condyle can be seen projecting well

reworked from older sediments. Hence Colbert⁶ in concluding his new interpretation of this supposed Jurassic labyrinthodont states "there will have to be more definite evidence than is afforded by the single specimen of *Austropelor wadleyi* to justify the extension of the labyrinthodont amphibians into Jurassic sediments". The discovery of the Kolane amphibian has provided the evidence that Colbert sought.

Not only does this specimen extend the range of the Labyrinthodontia to the upper Lower Jurassic but it also extends the occurrence of the Brachyopidae through the Middle and Upper Triassic, and the Lower Jurassic.

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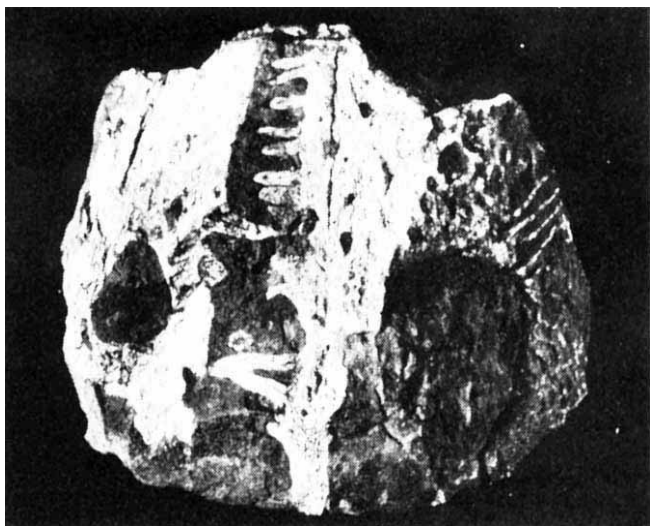


Fig. 1 Anterolateral view of part of the skull and lower jaw of the Jurassic labyrinthodont (Queensland Museum No. F7882). Part of the right orbit can be seen in the upper right hand section of the photograph. The scale measures 15 cm.

behind the level of the dorsal skull roof (Fig. 1). Orbits are well forward of the midline of the skull but are smaller than is usual in the brachyopids. The animal is big with its skull measuring more than twice the length and almost twice the breadth of the next biggest brachyopid, *Hadrokkosaurus bradyi* from the early Middle Trias³. A further link with that genus (which was also its closest relative in time) is that its vertebrae are of the rhachitomous type described by Welles and Estes³ in *Hadrokkosaurus*.

There has been one previous description of a Jurassic labyrinthodont. Longman in 1941⁷ described *Austropelor wadleyi*, a jaw fragment from the Marburg Sandstone, also of Queensland, Australia. While the age of this sandstone is probably equivalent to that of the Evergreen Formation, the fact that labyrinthodonts elsewhere in the world apparently became extinct at the end of the Triassic led de Jersey³ to postulate that this fragment must have been

Influence of temperature on cyanogenic polymorphisms

CYANOGENIC glucosides occur widely in the plant kingdom, and in species which are polymorphic for their presence or absence they probably have ecological significance. The cyanogenic morphs may be relatively unpalatable to grazing animals¹⁻³ and consequently have an advantage where grazing pressure is significant⁴. Cyanogenic morphs of *Trifolium repens* and *Lotus corniculatus* are relatively rare at high altitudes, and in Europe their distribution correlates with the January 5 °C isotherm⁵. It has been inferred that where low temperatures and frost are frequent, cyanogenesis confers a reduced physiological fitness, but this aspect of the polymorphism has received less attention and has not been established experimentally. A marked difference between the morphs in their response to artificial cold treatment is shown by the data reported here, together with evidence that this difference is significant in natural conditions.

Samples from the scarp slope of the Ochil fault line in the Forth Valley, Scotland, show the effect of altitude on the frequency of cyanogenic forms of *T. repens* and *L. corniculatus* (Table 1). At each site 100-150 plants were sampled, each a minimum of 1 m apart, and tested for the simultaneous presence of cyanogenic glucosides and β -glucosidase by the picrate paper test⁶. In both species the frequency of cyanogenics decreases as altitude increases; *L. corniculatus* is absent from the valley floor in this area. That it is selection which produces this distribution can be seen by comparing the seed and adult populations. *L. corniculatus* seed was collected at each site from 100 individuals, mixed and sown in garden plots. From each plot 250 individuals were selected randomly after 6 weeks growth and tested as before. Table 2 shows that in the highland site selection operates strongly against the cyanogenic morph and in the lowland site, to a lesser extent, against the acyanogenic morph. Selection probably operates through temperature, and so its effect was tested by placing individuals of both types at +5 °C and then lowering the temperature, over 2 h, to -3 °C. After 1 h at -3 °C plants

Table 1 Frequency of cyanogenic individuals at differing altitudes

Transect	Altitude (m)	<i>T. repens</i>		<i>L. corniculatus</i>		Transect length (m)
		Sample	% Cyanogenics	Sample	% Cyanogenics	
Sheriffmuir	312	100	2	107	47	4,000
	168	100	20	100	94	
	30	100	62	—	—	
Alva Glen	305	100	$\chi^2 = 94^*$	130	$\chi^2 = 54^*$	1,200
	106	100	5	160	67	
	15	100	83	—	100	
			$\chi^2 = 137^*$		$\chi^2 = 62^*$	

* $P < 0.001$.Table 2 Comparison of seed and adult populations of *L. corniculatus*

Altitude (m)	Seed		Adult		
	Sample	% Cyanogenics	Sample	% Cyanogenics	
312	250	86	107	47	$\chi^2 = 60^*$
168	250	79	100	94	$\chi^2 = 11^*$

* $P < 0.001$.

Table 3 Oxygen consumption as percentage of pretreatment level

Time from end of treatment (h)	Treated		Untreated	
	Cyanogenic	Acyanogenic	Cyanogenic	Acyanogenic
12	199 ± 21	139 ± 32	105 ± 12	101 ± 18
24	61 ± 31	93 ± 61	95 ± 18	90 ± 16
36	30 ± 14	79 ± 26	87 ± 18	105 ± 12
48	15 ± 15	102 ± 13	96 ± 12	93 ± 10

were removed to 20 °C for 30 min and tissue was sampled at 12-h intervals. The rate of respiration of excised tissue was measured in Warburg manometers, in the dark, at 25 °C. Oxygen consumption, measured as μl of oxygen per h per g dry weight for each 12-h period was expressed as a percentage of the oxygen consumption of the same plant in the 12 h preceding treatment. Control plants received identical treatment but were maintained at 20 °C. Table 3 gives the mean values, with standard errors, of three experiments in each of which different plants were used—12 individuals altogether. Damage resulting from the cold treatment caused a marked increase in the oxygen consumption of both types, but after 48 h acyanogenics were respiring normally whereas the respiration of cyanogenics decreased below the control levels within 24 h and continued to decrease thereafter. In all individuals the treatment caused the plants to wilt, but death of all above-ground shoots occurred only in some cyanogenics; acyanogenics recovered.

Selection therefore is shown to operate against cyanogenic forms at increasing altitudes, and temperature is probably the specific factor. The interplay of temperature effects with differential grazing probably maintains the polymorphism and governs the ecology of the morphs in some areas.

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Importance-value curves and diversity indices applied to a species-rich heathland in Western Australia

RANKING of species in a community from most to least important (abundant) to yield an 'importance-value' ($I-V$) curve is a device often used by ecologists to elucidate features of that community. Increasingly in the literature¹⁻⁵, an $I-V$ curve which obeys the lognormal function is being accepted as describing the ultimate in plant species diversity. South-western Australia is renowned for its apparent floristic richness and high degree of endemism. An ecological study of sand heath, considered by botanists the major repository of this supposed 'incredible richness'^{6,7}, has revealed a type of $I-V$ curve not previously noted for vegetation. The values for six of seven indices of species diversity examined were as high as, if not higher than, those for the most diverse communities previously recorded, including the richest of tropical rainforests^{2,4,8-10}. All earlier indices of equitability (evenness of species' importance) are based on models of limited applicability and a new index, based on the actual slope of the $I-V$ curve, is proposed here.

The study site, situated 15.5 km south of Eneabba (29°50'S, 115°15'E), was studied in mid-spring 1975. The vegetation was low open heath¹¹, consisting of nanophanerophytes (68%), hemi-cryptophytes (20.3%), chamaephytes (5.8%) and geophytes (5.8%)¹² and had been burnt about 7 yr before. According to Raunkiaer's leaf classes, 52.6% of the species were leptophylls, 26.3% nanophylls, 18.2% microphylls and 1% were mesophylls. The average growing season for agricultural plants in the area is 4 months. The soil was highly impoverished sand over yellow clay-loam at 50–60-cm depth in a shallow valley. A representative 1,500-m transect was run across the valley and 10 equi-spaced sectors, each consisting of an average of 50 contiguous 1-m² quadrats, were examined. The 13,066 individuals encountered were assigned to 172 species and their number and canopy cover class¹³ in each quadrat were noted. The total of 500 quadrats contained more than 96% of the species present

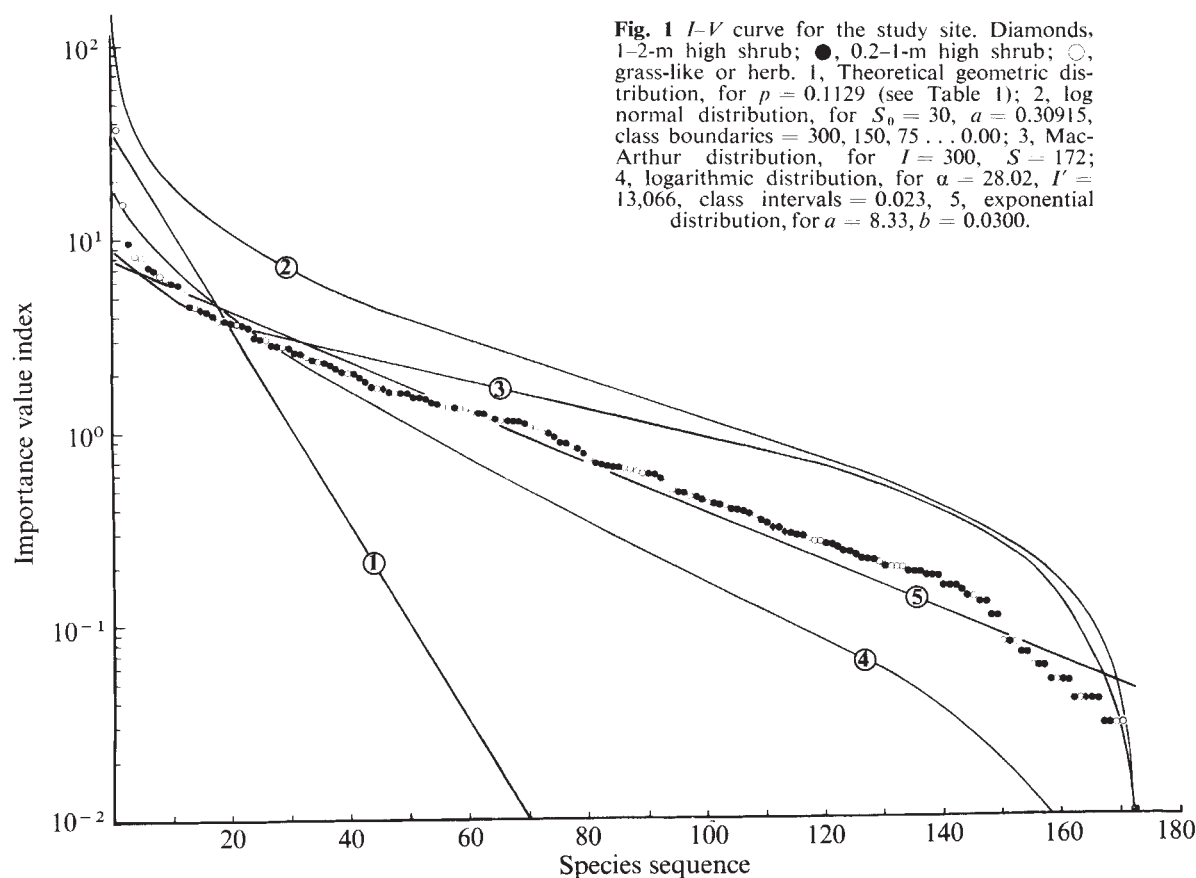


Fig. 1 I - V curve for the study site. Diamonds, 1-2-m high shrub; ●, 0.2-1-m high shrub; ○, grass-like or herb. 1, Theoretical geometric distribution, for $p = 0.1129$ (see Table 1); 2, log normal distribution, for $S_0 = 30$, $a = 0.30915$, class boundaries = 300, 150, 75 ... 0.00; 3, MacArthur distribution, for $I = 300$, $S = 172$; 4, logarithmic distribution, for $\alpha = 28.02$, $I' = 13,066$, class intervals = 0.023, 5, exponential distribution, for $a = 8.33$, $b = 0.0300$.

in a 150-ha area about the transect, though this low heath extends, with some taxonomic changes, for hundreds of km².

Species' importance was based on the importance value index (IVI) of Curtish and McIntosh¹⁴⁻¹⁶, as follows:

$$I_r = 0.1 \sum_1^{10} \left(\frac{100f_r}{\sum_1^{10} f_i} + \frac{100d_r}{\sum_1^{10} d_i} + \frac{100c_r}{\sum_1^{10} c_i} \right)$$

where I_r is the mean IVI of the r th species for the ten sectors, f is the frequency of occurrence in the 50 quadrats, d is the number of individuals per sector, c is the total canopy cover per sector and S is the number of species in the sector. From this formula, the total importance value (I) = $\sum I_i = 300$.

Though mathematically questionable, this measure was chosen to avoid bias in the exclusive use of any one index of abundance.

In Fig. 1, four mathematical distribution models often used to interpret empirical I - V curves have been superimposed on the data obtained for the study site. As expected from its low level of species' dominance, the vegetation had little in common with the geometric distribution¹⁷ ($P < 0.001$, Table 1). Though the lognormal curve¹⁸ had a similar shape to the I - V curve, the importance of any species was always considerably less than predicted by this distribution ($P < 0.001$) due to the leftward displacement of the modal interval. The presence of the complete untruncated curve using the lognormal model, however, confirms that the samples provided a good estimate of the number of species in the community¹⁹. The MacArthur

Table 1 Goodness of fit of various mathematical models to the I - V curve for the study site

Distribution	Formula	χ^2 value*	P	$D_{\max}^\dagger$	P
Geometric	$\hat{I}_r = I_1 (1 - p_1)^{r-1}$	∞	<0.001	0.4134	<0.001
Lognormal	$\Delta \hat{S}_R = S_0 / \exp \left(\frac{S_0 \sqrt{(\pi R)}}{S} \right)^2$	422.78	<0.001	2.0253	<0.001
MacArthur	$\hat{I}_r = \frac{I}{S} \sum_{i=1}^S \frac{1}{i}$	116.72	>0.05	0.1639 (I_1 omitted: 0.0853)	<0.001 >0.05
Exponential ($S_1 - S_s$)	$\hat{I}_r = a \exp(-bS_r)$	97.78	>0.05	0.1442	<0.001
Logarithmic	$\Delta \hat{S}_R = \alpha \left(\frac{I'}{I' + \alpha} \right)^{R-1} / R$	73.16	>0.05	0.0622	>0.05
Exponential ($S_{10\%+1} - S_{90\%-1}$)	$\hat{I}_r = a \exp(-bS_r)$	0.57	>0.05	0.0100	>0.05

$\hat{I}_r$ = estimated IVI for the r th species, $p_1 = I_1 / \sum I_i$, $\Delta \hat{S}_R$ = estimated number of species in an IVI interval R intervals from the modal interval with S_0 species, S = total number of species in the collection, a = constant (I_0), b = constant (slope), $S_r = r$, $\Delta \hat{S}_R'$ = estimated number of species in an IVI interval corresponding to R individuals, $\alpha = S / \log_e (1 + I'/\alpha)$ where I' = total number of individuals.

* χ^2 test on 170 d.f., $\chi^2_{0.05} = 201.14$, $\chi^2_{0.001} = 233.80$.

†Kolmogorov-Smirnov test: $D_{0.05} = 0.0959$, $D_{0.01} = 0.1191$, $D_{0.001} = 0.1343$ on 172 d.f.

distribution²⁰ gave a very close fit, particularly when I_1 was omitted ($P > 0.05$), though its 'characteristic' slope was flatter. Features in common between expectations from the MacArthur model and trends in the heath vegetation are a homogeneous habitat, species related taxonomically (taxocenes) and structurally (synusiae), and populations stable and long-lived (no annuals were present)—all suggesting lack of dominance and strong competition for resources⁵. Points of disparity are the views that the model is only applicable to limited numbers of animal species in communities of limited extent⁵.

By substituting number of individuals (I_i) for I_i as the measure of importance it was possible to consider Fisher's logarithmic distribution²¹. This gave the closest fit of the four classical curves ($P > 0.05$), though its 'characteristic' slope was steeper. The model assumes that additional individuals encountered include previously uncollected (rare) species in preference to previously collected (common) species, highlighting, in this case, the large number of species of low relative importance. The model implies a spatially unlimited community, such that further sampling would have included progressively more species which, as already stated, would not have occurred in the present case. Even in the final category ($I_i \rightarrow 1$, $I_i = 0.00-0.035$) there were five rather than the 28 species predicted by the logarithmic function. Though lacking a theoretical basis at present, the best fit to the 'characteristic' slope of the $I-V$ curve was provided by the truncated exponential distribution. Omission of the first and last 10% of species gave a near perfect fit (linear regression gives $r^2 = 0.992$), with implications for measures of diversity discussed below. The applicability of the total exponential curve was limited by its failure to change slope at the tails of the $I-V$ curve, though the fit was still close ($r^2 = 0.971$).

The reason for the poor fit of the lognormal curve may be that the heath vegetation was not as rich as expected. But using Gleason's index, based on the species-area curve²², the study site compared favourably with a tropical rain forest in Central Malaya ("... the richest community ever recorded")⁸ (Table 2). The figure for the rain forest was achieved with a much greater area (22.3 ha), far fewer individuals (2,629)—and hence a much higher Williams index²¹, and almost at the opposite end of the life-form spectrum. The inverse of Simpson's index²³, based on the geometric function, and the Shannon-Wiener index²⁴, based on the severe restrictions of information theory and tending to 'downplay' differences in species-rich communities⁵, had values in excess of a "species-rich" tropical

forest in the Panama Canal Zone ("... among the highest (H' values) ever reported for any community")⁹. The log-cycle equitability index⁵, based on the geometric model, was more than twice that for a swamp-heath with "a remarkably high species richness" in south-eastern Queensland¹⁰. Whittaker's equitability index⁴, based on the lognormal distribution, was more than four times that for the most diverse site along a 730-2,766 m gradient in south-eastern Arizona. Clearly, the present study site had 'surpassed' a lognormal relationship to reach a linear-sigmoid curve more related to the MacArthur or logarithmic models.

The restricted theoretical applicability of the usual measures of equitability described above, and the dissimilar slopes of the fitted theoretical distributions to that of the $I-V$ curve under study, indicate the need for an index that more truly reflects the characteristic slope of the $I-V$ curve and is not bound to particular community models. An inspection of 40 $I-V$ curves in the literature^{1,2,4,5,25,26} showed that, in addition to the present $I-V$ curve, all sets of data would fit an exponential curve, especially if the first and last 10% of data were omitted. The remaining 80% have a slope (b) which can be determined from $I_i = a \exp(-b S_i)$. Then the characteristic slope equitability index (E_b) is given by

$$\Delta S / \Delta \log_{10} \hat{f} = (S_{90\%+1} - S_{10\%+1}) / (\log_{10} \hat{p}_{10\%+1} - \log_{10} p_{90\%+1}) \\ = 1/0.434b$$

(see Tables 1 and 2 for the meaning of the symbols).

The arguments for omitting the first and last 10% of the data are essentially that they describe slopes that may be quite different from the characteristic slope; their component species are unlikely to be characteristic of (restricted to) that community, and their slope generally shows little variation between communities fitting quite different $I-V$ models. Indeed, a fuller description of the community might include the best fit lines of the three slopes, though this would seldom provide more information than E_b alone. Within the limits discussed here, the average concentration of importance for the first 10% of data ($\sum_{i=1}^{10\%} p_i^2 / S_{10\%}$) may be informative for certain purposes. On the same basis, the last 10% of data should indicate the degree of rarity of the least common species in the community. But the presence/absence of species in the last 10% is very dependent on sample size, some ecologists even being reluctant to quantify at this level^{26,27}.

Table 2 Values of various indices of species diversity for the study site and other species-rich communities

Index	Formula	Study site	Previous highest	Reference
Gleason's	$\frac{S}{\log_{10} A}$	64.10 (Sector ₁₀ = 69.2)	67.87	8
Williams	$\frac{S}{\log_e \left(1 + \frac{I'}{a}\right)}$	28.02 (Sector ₁₀ = 37.4)	114.2	8
Simpson's (inverse)	$\frac{1}{\sum_{i=1}^S p_i^2}$	38.77	34.48	9
Shannon-Wiener	$-\sum_{i=1}^S p_i \log_2 p_i$	6.12	5.86	9
Log-cycle	$\frac{S}{\log_{10} p_1 - \log_{10} p_S}$	48.73	23.10	10
Whittaker's	$\frac{S}{4\sqrt{(\sum_{i=1}^S (\log_{10} p_i - \log_{10} \bar{p})^2 / S)}}$	54.90	11.90	4
Characteristic slope	$\frac{S_{90\%+1} - S_{10\%+1}}{\log_{10} p_{10\%+1} - \log_{10} p_{90\%+1}}$	86.73	≈ 41.64	2

See Table 1 for meaning of symbols. In addition, A = area in m², $\log_{10} \bar{p} = \sum \log_{10} p_i / S$, $p_{10\%+1}$ = estimated p (or I) for the (10%+1)th species from the truncated exponential curve fit in Table 1.

Using Whittaker's criteria of a good diversity index⁵, E_b can be considered relatively independent of sample size—rare species do not influence the slope, and the effect of any over-estimation of importance of dominants in small samples is minimised. Sampling error is low—80% of the species collected contribute to the index, standard regression analysis can be used to determine the level of sampling error and dispersion about the mean is minimised by removing the tails. E_b is conceptually meaningful—it is literally (the inverse of) the slope of the best fit line; the numerator is based on the total number of species and hence the formula also gives the relationship between richness and equitability. It is suitable for any taxocene or measure of importance, and its inverse-slope character enhances its sensitivity to changes in evenness.

E_b for the study site was more than twice that for a naturally regenerated deciduous forest in southern Illinois of "exceptionally high species diversity"². Unlike the latter site, there was little evidence of vertical heterogeneity or creation of micro-habitats in the heath vegetation that could explain its much greater diversity. Certainly the small life forms, open canopies and similar resource requirements would encourage close packing of these slow-growing perennials with minimal opportunity for dominance. This would be enhanced by proneness of the site to fire, drought and severe leaching. In addition, the site's location in a conjectured 'centre of diversification' of the south-western flora²⁸—45% of the species belonged to a mere three families—would provide a large pool of coloniser species. Assuming that fluctuations about the present climate have occurred here in the past, this proposition is consistent with Stebbins' argument²⁹ that speciation is most favoured in transition zones between xeric and mesic or tropical environments.

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Reciprocal altruism in *Papio anubis*

ALTRUISM is behaviour that benefits another individual at some cost to the altruist, costs and benefits being measured in terms of individual fitness. 'Reciprocal altruism' (ref. 1) implies the exchange of altruistic acts between unrelated individuals as well as between relatives. If the benefits to the recipient of an altruistic act exceed the costs to the altruist, and if the recipient is likely to reciprocate at a later time, then the cumulative benefits for both individuals will have exceeded the cumulative costs of their altruism. Natural selection would favour individuals that engaged in reciprocal altruism if they distributed their altruism with respect to the altruistic tendencies of the recipient, preferring individuals that were most likely to reciprocate and excluding nonaltruists from the benefits of further altruism. This model has been difficult to test because it is usually impossible to be certain that an example of altruism is not the product of 'kin selection'². The genetic relationships between individuals in animal populations are seldom known and reciprocal altruism can only be cited when it can be found to occur regularly between unrelated individuals. I report here that altruistic behaviour involving the formation of coalitions among male olive baboons (*Papio anubis*) fulfils the criteria for reciprocal altruism.

Eighteen adult male *P. anubis* in three troops at Gombe National Park, Tanzania were studied for more than 1,100 h, between May and December 1972 and from June 1974 to May 1975. All data were collected on a focal sample basis³. Focal individuals are referred to as 'targets'. Each animal was observed regularly for a fixed period. Observations were made on foot at 5–10 m from the animal. Baboon studies have been in progress since 1967 and data concerning blood relationships and dates of transfer between troops are available. All males leave their natal troop at Gombe, and the males who breed within a particular troop are those that have transferred into that troop from elsewhere⁴. Males known or thought to have transferred into their troop of residence are termed 'adult males'. Sexually mature males still residing in their natal troop are 'natal males'. During the study, there were three, six to eleven, and eight to eleven adult males in the respective study troops.

The effectiveness of temporary coalitions of adult male *Papio* spp. during aggressive interactions against a single opponent has been described in *P. anubis*⁵ and in *P. ursinus*⁶. Encounters between coalitions and single opponents in *P. cynocephalus* did not seem to affect subsequent dyadic encounters between a coalition member and the opponent⁷. Ransom noted that coalitions of *P. anubis* at Gombe were generally formed by one male enlisting a partner to help fight against an opponent⁸. The partner had not been involved directly in the encounter with the opponent before his enlistment. Coalitions were sometimes formed in attempts to separate an opponent from an oestrous female. *P. anubis* form exclusive consort pairs lasting for up to several days. If the female became available after such an attempt, she could be taken by only one of the two coalition partners.

Attempts at enlisting a coalition partner are referred to as 'soliciting'—a triadic interaction in which one individual, the enlisting animal, repeatedly and rapidly turns his head from a second individual, the solicited animal, towards a third individual (opponent), while continuously threatening the third. The function of headturning by the enlisting animal is to incite the solicited animal into joining him in threatening the opponent. An 'occasion' of soliciting is a bout of the behaviour followed by a gap of more than 10 s. The distribution of the number of 'occasions' of soliciting to the same partner per observation period was tested against a cumulative binomial distribution and did not differ significantly from expected values. Therefore, 'occasions' of soliciting are considered to be statistically independent from each other. For every occasion that soliciting

involved the target male during an observation period, the identity and actions (enlisting, solicited, or opponent) of each participant were recorded. 'Occurrences' were not recorded consistently in 1972, so data from that period are not included in measures of frequency. Although coalitions occasionally formed spontaneously, only those that resulted from soliciting are considered.

There were 140 examples of one adult male soliciting another during the 1974-75 study period. Soliciting resulted in coalitions on 97 occasions. On 20 occasions the opponent was consorting with an oestrous female and adult males were more likely to join a coalition if the opponent was in consort ($2 \times 2 \chi^2 = 5.91$, $P < 0.02$, $n = 140$). On six occasions during both study periods the formation of a coalition directed against a consorting male resulted in the loss of the female by the opponent. In all six cases the female ended up with the enlisting male of the coalition ($P = 0.032$, two-tailed, sign test); the solicited male generally continued to fight the opponent while the enlisting male took over the female. In each of those cases the solicited male risked injury from fighting the opponent while the enlisting male gained access to an oestrous female. In most other examples of soliciting, no resource appeared to be at stake. The greater willingness to join a coalition against a consorting male may be related to the greater benefits that the altruism bestows on the recipient in those cases.

Thirteen different pairs of males reciprocated in joining coalitions at each other's request on separate occasions. These pairs comprised 12 different males. In six of these pairs, both pair members successfully enlisted their partner against the same opponent. Individual males which most frequently gave aid were those which most frequently received aid. There was a strong correlation between the frequency with which adult males joined coalitions and the frequency with which they successfully enlisted coalition partners ($\hat{\rho} = 0.84$, $z = 2.87$, $P < 0.004$, Kraemer test⁹ based on Spearman correlations).

Each male tended to request aid from an individual who in turn requested aid from him. Using only one occasion of soliciting per pair per observation period (since spatial patterns are relatively stable), the distribution of soliciting by each of the 18 target males was examined to find which other male each target solicited most often, that is, his 'favourite partner'. Only the ten targets who solicited other males on four or more occasions were included. After the favourite partner was found for each target male, the distribution of soliciting by each partner was similarly examined. For nine out of ten target males, the favourite partner in turn solicited the target male more often than the average number of occasions that the partner solicited all adult males in their troop ($P = 0.022$, two-tailed, sign test) (Table 1). These results suggest that preferences for particular partners may be partly based on reciprocation.

It is difficult to test whether a non-altruist is excluded from further altruistic exchanges; a refusal to join a coalition in any given instance may occur because the costs to the potential altruist on that occasion are particularly high. There is evidence, however, that adult males are more likely to join coalitions with individuals which in turn would be able to help them. Females and juveniles ('non-males') solicited adult males 12 times, but adult males never solicited 'non-males' ($P < 0.001$, two-tailed, sign test). Adult males were less likely to respond to the solicitings of 'non-males' than they were to other adult males ($2 \times 2 \chi^2 = 7.76$, $P < 0.01$, $n = 152$), even though 'non-males' solicited adult males against other 'non-males' far more often than adult males solicited other adult males against 'non-males' ($2 \times 2 \chi^2 = 43.76$, $P < 0.001$, $n = 110$). Adult male baboons are very much larger than either adult females or juveniles, so that even though the aid of an adult male to a 'non-male' would generally incur small costs to the adult male, the benefits to the male from having a 'non-male' partner would be trivial, since a 'non-male' could not provide effective help in an encounter against another adult male and an adult male would not need help in fighting a 'non-male'.

The adult males in the troops studied transferred into their troop of residence singly¹⁰. The origins of many males entering a study troop were not definitely known, so it was impossible to determine the precise degree of relatedness between most adult males. The previous troop and dates of transfer of each male were known, however. In 4 of the 13 reciprocating pairs, both partners were first observed as young adults in different troops when they probably had not yet transferred for the first time. They did not reside in the same troop for another 5 yr. One pair not included earlier comprised an adult male and a 'natal' male which were known to have been born in separate troops. Although there is no proof that any of these individuals are completely unrelated, it is unlikely that they are close relatives.

Table 1 Soliciting activity of favourite partners of target males

Target	No. of occasions FP solicited target	Mean no. of occasions FP solicited each male	FP solicited target more; + or less; - than average
BBB	3	2.7	+
CRS	3	1.2	+
DVD	0	1.0	-
EBN	5	1.0	+
GRN	1	0.7	+
JNH	2	1.1	+
LEO	4	2.7	+
MNT	1	0.4	+
WDY	5	2.7	+
WTH	4	2.8	+

FP, favourite partners.

In contests between a coalition and an opponent a previously uninvolved partner may benefit the enlisting male by reducing the latter's risk of injury in fighting the opponent. (Although only one male has been known to die from wounds received in a fight since 1967, non-fatal wounds are common.) By participating in a fight in which he would not have been otherwise involved, his aid will have been at some cost to himself. Whether or not the benefits to the recipient exceed the costs to the altruist (as required by the model) is difficult to determine. But, for the opponent, the potential costs in facing two males simultaneously rather than only one might be so much greater that it would often be to his advantage to avoid the coalition without fighting. If so, then the actual costs to the altruist are less than the reduction in costs to the enlisting male since the enlisting male would then be less likely actually to fight the opponent. When the formation of a coalition involves gaining access to an oestrous female further benefits are involved. Coalitions are the most common way of aggressively taking over an oestrous female from a consorting male at Gombe (D. A. Collins, in preparation). During the reproductive life of a male, which extends over 10 yr, there may be a large number of situations where it would be advantageous to enlist a coalition partner in an encounter against a consorting male. The number of offspring that a male sired as a result of participating in reciprocating coalitions would be greater than if he did not, while his lifespan would probably not be appreciably shortened by aiding coalition partners.

There are probably occasions when altruism is not involved. For example, the solicited male may sometimes join a coalition when there is a prospect of immediate benefit to himself. In such cases, explanations of the animal's behaviour are not necessary; the behaviour is not detrimental to his own fitness. The occurrence of genuine altruism, however, seems to be common enough to demand an alternative explanation.

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Sound as a host-detection cue for the soft tick *Ornithodoros concanensis*

Two parasitic flies are attracted to their invertebrate hosts by sound: a tachinid, *Euphasiapteryx ochracea*, is attracted to field crickets by their song¹ and a sarcophagid, *Colcondamyia auditrix*, orients acoustically to cicada song². We now report that an acarine, the argasid tick *Ornithodoros concanensis*, makes use of the vocal sounds of a vertebrate host as a cue in its host-finding behaviour.

Recorded vocalisations of nestling cliff swallows and electronically generated sounds of specific frequencies were used to test responses of unfed and engorged nymphs of *Ornithodoros concanensis*. Specific frequencies and their pattern of modulation were selected for testing after sonographic analysis of the recorded nestling vocalisations. Dominant sounds produced in the calls were in the 3,000–8,000-Hz range. Three frequencies (3,000, 5,200 and 8,000 Hz) prevalent in the nestling vocalisations and additional sounds above (12,000 and 15,000 Hz) and below (500 and 1,000 Hz) these levels were selected for testing. During experiments the generated sounds were modulated to a pulse repetition rate of one per s and a duration of 140 ms to approximate the pattern of nestling vocal emissions. Sound intensities for all generated frequencies and the recorded nestling sounds graded down from 62–66 dB on the edge of the stage proximal to the speaker (8 cm from the speaker) to 61–64 dB at the centre (19 cm from the speaker) to 60–62 dB at the distal edge of the stage (30 cm from the speaker). Control experiments were done with the speaker turned on but without taped bird sounds or output from the oscillator. The stage consisted of a circular paper grid (22 cm diameter) on a piece of 2.5-cm thick styrofoam. The loud speaker was positioned 8 cm from the stage and its base rested on a 2-cm thick polyurethane foam pad. The stage and speaker were enclosed within a rectangular sound arena (69 cm long, 24 cm wide and 31 cm high) constructed of 10-cm thick porous polyurethane foam. A diffuse light centred 150 cm above the stage generated light of less than 0.22 mW cm⁻² on the stage surface. During trials the temperature on the stage was 26.6 ± 1 °C and the relative humidity ranged from 15 to 18%. Before use in an experiment, ticks in individual vials were maintained for at least 6 weeks after feeding at 85% relative humidity, 25 °C, and in a 24-h light cycle with 14 h of light. Trials were begun 2 h after the onset of darkness. To initiate a trial the vial containing a tick was inverted gently and the tick was allowed to fall on to the centre of the stage. Tick movements were observed and the point at which each tick left the circular stage was recorded on a data sheet. At the conclusion of a trial a data-set resembled one of the plots in Fig. 1. The mean number of ticks used in each trial was 22. The numbers tested at each frequency and in controls are shown in Table 1, together with a summary of statistical analysis. Rayleigh's test³ was used statistically to evaluate whether the circular dispersion of the ticks was non-random.

In experimental trials unfed nymphs demonstrated a significantly pronounced movement toward the speaker when the

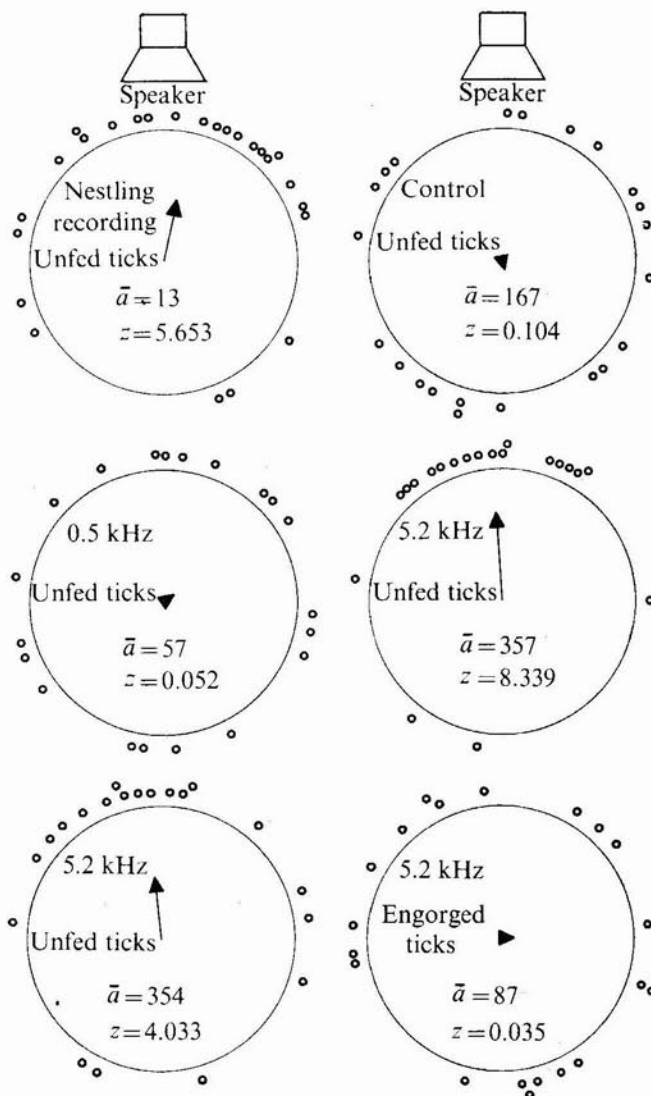


Fig. 1 Selected examples of the dispersal of ticks illustrate their tendency to move toward a speaker generating specific sounds. The small circles represent the positions to which ticks moved after being released in the centre of the circular stage. Symbols relating to the circular distribution analysis are: $\bar{a}$ = the mean angle vector; z = Rayleigh's critical value; and the length of the vector indicates the amount of relative circular dispersion of the group of ticks. Tape recordings of nestling cliff swallows were made with a Panasonic tape recorder (RQ-209-DAS) and a Panasonic dynamic microphone. The tape recordings were analysed with a Kay sonograph 6061A spectrum analyser. Specific sound frequencies were generated with a Hewlett-Packard Model 200 CDR wide range oscillator, a Grass S-5 stimulator, and a Grass AM-4 a.c. audio monitor. The Oxford loudspeaker (8.9-cm, 3.2 Ω) that was used has a frequency response of 50–15,000 Hz. Light intensity was measured by a Weston Model 76 illumination meter. Sound intensities were measured with a Type 2203 Bruel and Kjaer precision sound level meter.

recording of nestling sounds was played and when the frequencies within the nestling sound range (3,000, 5,200 and 8,000 Hz) were generated. Such movements were not seen with trials involving 500, 1,000, 12,000 and 15,000 Hz nor in control trials of unfed ticks (Table 1). After being released on the centre of the stage, unfed ticks characteristically would remain akinetic for 3–5 min. Positively responding ticks would then orient toward the sound source before proceeding forward with movements involving wavy or zigzag deviations from a straight line. From our results it was not possible to determine whether the pattern of orientation is a klinotaxis or a tropotaxis. A few ticks responded with pronounced turning suggestive of a klinokinesis. During trials involving the sounds to which ticks responded positively, it often seemed that the first pair of legs contracted in synchrony with each sound pulse from the speaker. By directing the modulated sounds of specific

Table 1 Analysis of tick responses to generated sound frequencies and recorded host vocalisations

Frequency (Hz)	Experimental trials		Controls	
	No. of ticks	Prob-ability	No. of ticks	Prob-ability
Unfed				
500	21	NS	20	NS
1,000	19	NS	20	NS
3,000	23	$P < 0.001$	20	NS
5,200	20	$P < 0.001$	20	NS
8,000	30	$P < 0.05$	22	NS
12,000	21	NS	22	NS
15,000	20	NS	22	NS
Nestlings	25	$P < 0.005$	22	NS
Engorged				
5,200	20	NS	20	$P < 0.02$

The null hypothesis of a random distribution of ticks around the circle was accepted and the trial results were considered not significant (NS) if the probability was greater than the 0.05 level.

frequencies (for example, 3,000 Hz) at a tick being observed through a dissecting microscope, it was possible to confirm twitching or contracting of the first pair of legs with each sound pulse. This suggests a possible location of sound receptors in these appendages.

Immediately after observing a positive response of one group of unfed nymphs to 5,200 Hz, the ticks were fed on pigeons and individually retested at the same frequency. In contrast to the unfed ticks, engorged individuals were not temporarily akinetic when introduced to the stage, but immediately began to move. The pattern of dispersal of the engorged ticks was random.

Ornithodoros concanensis is a parasite of cliff swallows (*Petrochelidon pyrrhonota*), other birds and bats⁴ associated with cliff and cave habitats in western North America. Concealed in the cracks and crevices during the day, the ticks move up the cliff face at night to obtain blood meals from cliff swallows roosting in their retort-shaped nests. Bird activity diminishes sharply at sunset but vocalisation in the nest continues during the night, particularly while young birds are present. Host-finding activity of unfed ticks begins 30 min to 1 h after sunset (approximately 2100 h), peaks 1–2 h later, and ebbs by 0100 or 0200 h. Experiments have indicated that nymphal *O. concanensis* are not stimulated by elevated concentrations of carbon dioxide that approximate normal (4–5%) or below normal levels of host-breath emission. Furthermore, host odour failed to elicit positive reactions and heat was important only at close range⁵. All indications are that sound is the principal cue used by *O. concanensis* for orientation to and location of host cliff swallows. After a blood meal is obtained the bird sounds are no longer attractive to the engorged tick and a combination of stimuli influences its movement out of the nest and back into a harbourage.

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Evidence for role of flagella as sensory transducers in mating of *Chlamydomonas reinhardtii*

FLAGELLA and cilia are motility organelles of many cells. Modified cilia are present in most metazoan sensory cells¹, and mechanosensory transduction by cilia is found in many protozoans and some invertebrates². The question arises as to whether flagella used primarily for motility also have sensory functions. In the unicellular alga *Chlamydomonas reinhardtii*, flagella act as organs of motility. It has been observed, however, that flagellar contact initiates the mating process when gametes of opposite mating types are mixed^{3–6}. We have been studying this phenomenon and report here observations on the role of flagella in initiating the mating reaction.

The mating process commences following the mixing of (+) and (–) gametes of *Chlamydomonas reinhardtii*. Several physiological changes have been observed. These include cell wall lysis^{4–6} and fertilisation tubule formation in the (+) gametes^{3,6}. We have previously reported the accumulation of carbohydrates in a cell-free supernatant of mating cells, probably a result of cell wall lysis. The accumulation is specific and occurs only when live gametes of opposite mating types are mixed⁷.

As shown in Fig. 1, there is a 45-min delay in carbohydrate accumulation if the gametes are deflagellated at the time of mixing. Flagellar regeneration in *Chlamydomonas reinhardtii* has been well studied^{8–11}, but not in a mixture of

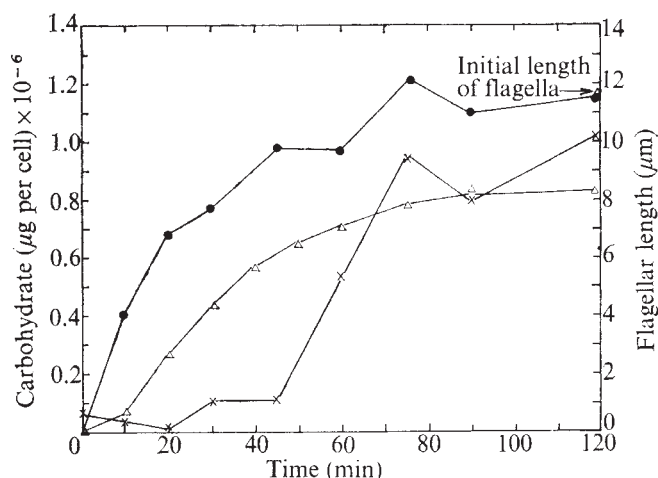


Fig. 1 Carbohydrate release and flagellar regeneration. *Chlamydomonas reinhardtii* were grown as described before⁷, except that they were on a 12-h light–12-h dark cycle. At time 0, active (+) and (–) gametes were mixed. The flagella of the experimental group were detached upon mixing using the pH method¹². Deflagellation was accomplished by lowering the pH to 4.5 for 1 min with 10% acetic acid and neutralising with KOH. Detachment was followed with phase microscopy and more than 99% of the cells became deflagellated. Flagellar regeneration was observed in at least 90% of the cells which were deflagellated. The non-deflagellated control group had acetic acid and KOH added simultaneously. Samples of the cell suspension (1.6×10^7 cells) were taken at intervals and carbohydrate analysed in the cell-free supernatant as described before⁷. Flagellar regeneration was followed using the methods of Rosenbaum *et al.*⁹. It is interesting to note that after 2 h of regeneration, the flagella had reached only about 75% of their original length. For vegetative cells it has been reported that the flagella are 90% regenerated after 60 min⁹. We attribute the discrepancy to the fact that in our case, the cells are mating, and that during the actual cell fusion flagella grow slowly or not at all. ●—●, (+) and (–) gametes, non-deflagellated control; ×—×, (+) and (–) gametes, flagella detached upon mixing; △—△, flagellar length in μm. Each point represents the average of two experiments.

mating gametes. To ascertain the relationship between flagellar length and initiation of mating, we monitored flagellar regeneration in a population of deflagellated mixed gametes. As indicated by the carbohydrate accumulation (Fig. 1), the mating reaction commences after the flagella have regenerated to more than 50% of their original length. Cells regain motility 25 min after deflagellation, and at this point the flagella are about 25% of their original length. There is, therefore, a delay of at least 20 min after the return of motility before carbohydrate accumulation can be detected. Thus, it seems that the limiting factor for mating is the length of the flagella, and that some sort of flagellar differentiation occurs when the organelles are about 50% regenerated. This maturation allows the organelle to act as a sensory transducer for the mating reaction.

An alternative explanation involves the lowered probabilities of gametic interaction when flagella are shorter than normal. This explanation is unlikely, however, for two reasons. First, the cells are at a very high concentration (2×10^7 cells ml^{-1}). Second, the 45-min delay in mating, as measured by carbohydrate accumulation, is present even if only one of the mating types is deflagellated and mixed

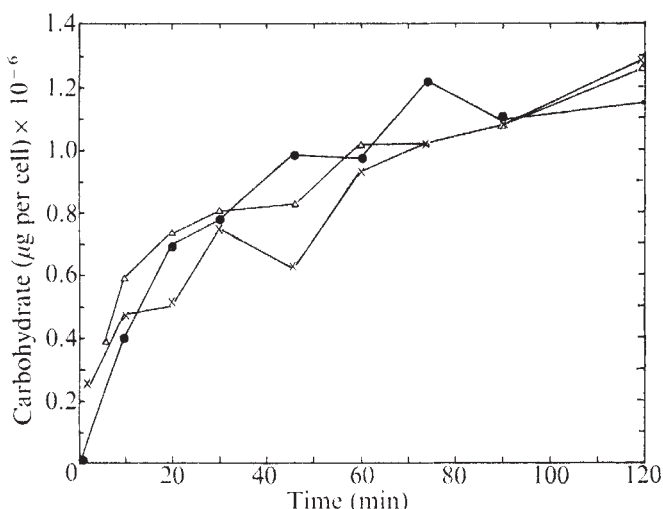


Fig. 2 Carbohydrate release and detachment of flagella. Conditions of cell growth, flagellum detachment and carbohydrate analysis are the same as in Fig. 1. At time 0, active (—●—) and (—) gametes of *Chlamydomonas reinhardtii* were mixed. At 2 min and 4 min after mixing, aliquots were taken and deflagellated. At intervals thereafter, samples of the cellular suspension (1.6×10^7 cells) were taken and analysed for carbohydrate as previously described⁷. ●—●—●— (—) and (—) gametes, non-deflagellated control; ×—×—×— (—) and (—) gametes, flagella detached 2 min after mixing; ○—○—○— (—) and (—) gametes, flagella detached 4 min after mixing. Each point represents the average of two experiments.

with the opposite mating type. This implies that in spite of the increased likelihood of encounter resulting from the presence of full length flagella in one of the mating types, mating did not occur until the flagella from the deflagellated cells attained 50% of their normal length. The crucial finding is that the time delay in this situation was identical to the delay following deflagellation of both mating types.

Figure 2 shows the effect of deflagellation at various times after mixing the gametes. It can be seen that cells deflagellated at either 2 min or 4 min after mixing continue in the normal time course of carbohydrates accumulation. Since the deflagellation procedure (a quick pH change—see Fig. 1) does not in itself induce the gametes, the lack of a delay in carbohydrate accumulation indicates that the flagella act as transducers within the first 2 min of mating.

From our data we can conclude that a signal responsible for initiating the mating process in the gametes of *Chlamydomonas reinhardtii* is transmitted by means of the

flagella. Without flagella that induction is delayed for 45 min, when the flagella have regenerated to more than one half their original length. This form of sensory transduction occurs within the first 2 min of mating, since the loss of flagella after that point does not inhibit the mating reaction as measured by carbohydrate accumulation. The reaction seems to be associated with the 'mature' tips of the flagella, and because of its specificity we assume it to be chemical. At this point, however, we cannot be specific as to the actual mechanism of the reception and transduction of the mating signal. The significance of this work lies in the fact that we have been able to demonstrate that the flagella of *Chlamydomonas reinhardtii* are both organelles of motility and sensory transducers in the mating reaction.

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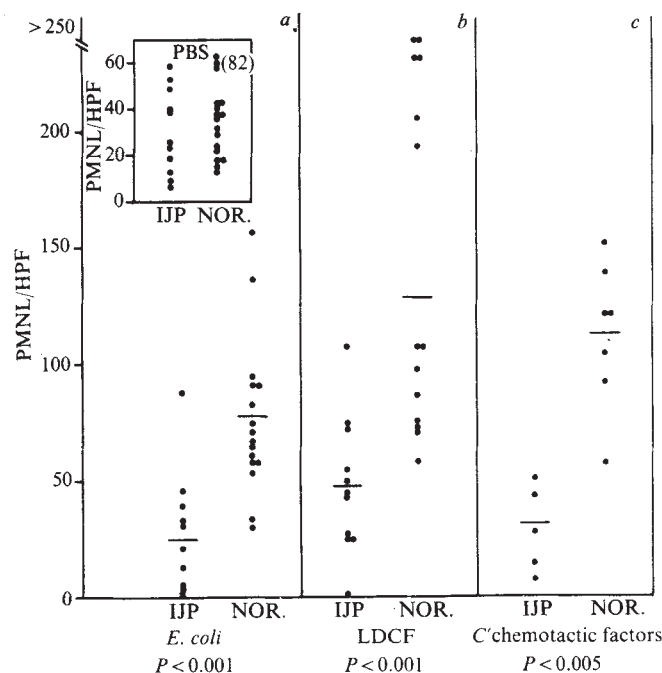
Defective polymorphonuclear leukocyte function in a human periodontal disease

HUMAN periodontal diseases are the major cause of adult tooth loss. One such disease, localised idiopathic juvenile periodontitis (IJP) affects young people and is unusually severe, with a rapid progression. Previous studies have shed little light on the unusual susceptibility of some patients to this rare condition. Similarly, destructive periodontal syndromes have been described in patients with polymorphonuclear leukocyte (PMNL) dysfunction. We have measured two parameters of neutrophil function, chemotaxis and phagocytosis, and found that patients with IJP have significantly decreased PMNL function compared with healthy control subjects, but monocyte function remains unaffected in IJP. Reduced PMNL functions may contribute to the unusual susceptibility of patients to IJP.

Periodontal diseases are progressively destructive inflammatory processes affecting the supporting tissues of the teeth. By age 45 yr almost all individuals have periodontal disease; under age 45, a smaller but substantial part of the population is affected¹. As periodontal diseases are accessible to study, they are excellent models for the investigation of host-parasite interactions in bacterial inflammatory disease.

Studies of IJP by many investigators have failed to establish any specific etiology² or account for the unusual susceptibility of certain individuals. A specific alteration in the host's protective response could explain the rapidly destructive character of this disease, but no major systemic involvement in such patients has been observed regularly. Therefore, any impairment in the host's protective response is likely to be subtle. Study of parameters of host resistance in patients with IJP, by sensitive procedures, may reveal mild alterations in protective response.

PMNL have been implicated in the inflammatory lesions of periodontal disease^{3,4}. Patients with PMNL disorders such as cyclic neutropenia⁵, agranulocytosis⁶, and Chediak-



an atmosphere of 5% CO₂ in humid air. The filters were then removed, stained, mounted on slides and viewed in a microscope. Cells migrating to the lower surface of the filter in 20 oil immersion fields were counted. Cell migration was expressed as the mean number of migrated cells per high-power field (HPF) for quadruplicate samples minus the mean background number of cells per field migrating towards a non-chemotactic (PBS) control. Data from IJP and normal subjects were grouped and compared by the Mann-Whitney U test. Horizontal bars represent the arithmetic mean for each group.

Higashi disease⁷ show unusually severe loss of the supporting alveolar bone of the teeth. This may result from defective PMNL defence against oral bacteria. *In vitro* assays have shown leukocyte function to be reduced in many diseases. For example, Snyderman⁸ has shown a defect in mononuclear leukocyte chemotaxis in patients with chronic mucocutaneous candidiasis. Defective PMNL chemotaxis is also associated with many disease conditions^{9,10}. Gillman *et al.* reported a positive correlation between reduced neutrophil phagocytosis and susceptibility to middle ear infections in otherwise healthy individuals¹¹.

The subjects of our study were four males and five females in whom IJP was diagnosed as defined by the criteria of Kaslick²; three of the females were sisters. Diagnostic criteria, established by clinical and radiographic evaluation, included severe localised alveolar bone loss around the first molars and incisors of both maxillary and mandibular permanent teeth. Patients were 16–31 yr old (average 20.0 yr). Health status was established by a thorough medical history, a physical examination, and a battery of laboratory tests. The results of the tests were always within established norms, and peripheral blood PMNL numbers were not different from those of a panel of normal subjects. Leukocyte differential counts were also within the normal range. All patients had a negative history of frequent or unusual illnesses and seemed in good general health.

Chemotaxis of PMNL and monocytes, and phagocytosis by PMNL were measured. Peripheral blood leukocytes were assayed simultaneously from patients with IJP and from normal subjects of similar age, sex and race. Samples from those with IJP were assayed on at least two separate occasions to demonstrate reproducibility of the observations. Chemotaxis was assayed using the modified Boyden chamber technique described by Snyderman¹², and phagocytosis was measured by the procedure of van Oss and Stinson¹³.

When tested with *Escherichia coli* culture filtrate, leukocyte-derived chemotactic factor, and human

Fig. 1 Chemotactic response of PMN leukocytes (PMNL) from individuals with IJP compared to normal (NOR.) individuals. *a*, *E. coli* 0127 was grown in trypticase soy broth for 18 h at 37 °C. The bacteria were removed from suspension by centrifugation and the culture filtrate was passed through a 0.20-μm filter. *b*, Leukocyte-derived chemotactic factor (LDCF) was prepared from a 48-h culture supernatant fluid of phytohemagglutinin-stimulated human peripheral blood mononuclear leukocytes. *c*, Chemotactic components of complement¹⁸ were prepared by incubation (for 30 min at 37 °C) of 0.1 ml of fresh human serum with 0.9 ml of gelatin veronal buffer and 0.1 ml of a 1-mg-ml⁻¹ suspension of lipopolysaccharide derived from *Salmonella typhimurium* (Difco). Each chemotactic preparation was divided into aliquots, used fresh or stored at -76 °C until used. The chemotaxis assay was as described by Snyderman *et al.*¹². On any one day, responder cells prepared from peripheral blood of at least one individual with IJP were compared with responder cells prepared from one or more normal, age-matched subjects. Leukocytes for each experiment were separated from erythrocytes by dextran sedimentation of whole blood. Mononuclear cells and neutrophils were then purified from the leukocyte-rich plasma by Ficoll-Hypaque gradient centrifugation. The neutrophils in the pellet were washed twice in 0.15 M NaCl buffered with 0.02 M sodium phosphate at pH 7.2 (PBS), and diluted with Gey's balanced salt solution (pH 7.2) to 2.5 × 10⁶ cells per ml (personal communication from L. C. Altman). The neutrophils were placed in the top compartments and the chemotactic preparations or PBS (non-chemotactic negative control) were placed in the bottom compartments of modified Boyden chambers. The compartments were separated by a polycarbonate filter with 3-μm diameter pores (Nuclepore, Wallabs). The chambers were incubated for 30 min at 37 °C in

complement-derived chemotactic factors¹², the polymorphonuclear leukocyte chemotactic response of patients with IJP was significantly less ($P < 0.005$) than that of normal subjects (Fig. 1). By contrast, the chemotactic response of peripheral blood monocytes of IJP patients did not differ from that of the normal subjects. No differences were seen between the groups in background PMN or monocyte cell migration against the non-chemotactant saline control.

Phagocytosis of opsonised *Staphylococcus aureus* by PMNL from patients with IJP and normal subjects was then compared (Table 1). All the subjects with IJP had depressed PMN phagocytosis as compared with normal controls. The contact angles formed by sessile drops of saline water on monolayers of PMNL (see refs 14 and 15) of patients and normal controls are also compared in Table 1. A higher contact angle, and thus a less hydrophilic surface of phagocytic cells is invariably accompanied by a decrease in phagocytic activity¹⁵. The differences in phagocytosis as well as in contact angles are significant at the 95% confidence level or greater with one exception. Leukocytes of two young patients with generalised periodontitis, clinically distinct from IJP, showed normal responses in assays for chemotaxis and phagocytosis.

Thus there is a reduction in both chemotaxis and phagocytosis by PMN leukocytes in patients with IJP, although monocyte chemotaxis is normal. In assays of the peripheral blood leukocyte blastogenic responses to phytohaemagglutinin and streptolysin O in these IJP patients, no obvious defects were found. More discriminating tests are in progress to assess cellular immunity more fully.

Lavine *et al.*¹⁶ studied the response of PMNL from patients with early onset periodontitis to *E. coli*-derived chemotactic factor, and concluded that PMNL from these patients are characterised by an intracellular defect in chemotaxis unrelated to serum inhibitory factors. This disease entity was probably IJP, providing further evidence that such PMNL have defective chemotaxis.

Newman *et al.*¹⁷ noted several distinct anaerobic Gram-negative rods, exhibiting surface translocation which seemed

Table 1 Phagocytic activities and contact angles of neutrophils of patients with IJP and of normal controls

	IJP			Normal controls	
	Phagocytic activity*	Contact angle $\pm$ s.e.		Phagocytic activity*	Contact angle $\pm$ s.e.
LB	5.5 $\pm$ 1.2	19.1 $\pm$ 0.3°	CD	9.6 $\pm$ 2.3	17.7 $\pm$ 0.2°
VB	4.4 $\pm$ 1.4	19.6 $\pm$ 0.5°	MI	8.3 $\pm$ 1.0	17.8 $\pm$ 0.3°
GB	4.2 $\pm$ 0.5	19.4 $\pm$ 0.5°	BR	11.7 $\pm$ 2.1	18.1 $\pm$ 0.4°
HO	7.5 $\pm$ 0.5	19.6 $\pm$ 0.5°	LP	10.1 $\pm$ 1.2	18.3 $\pm$ 0.3°
VA	2.9 $\pm$ 0.6	18.9 $\pm$ 0.5°	BL	9.1 $\pm$ 1.3	18.3 $\pm$ 0.3°
HU	4.8 $\pm$ 0.6	19.4 $\pm$ 0.4	CI	9.3 $\pm$ 0.7	17.6 $\pm$ 0.2°
AD	3.6 $\pm$ 0.4	20.0 $\pm$ 0.4	PE	10.0 $\pm$ 0.9	17.5 $\pm$ 0.1°
YO	4.6 $\pm$ 0.5	20.4 $\pm$ 0.4			
FI	6.1 $\pm$ 0.7	19.2 $\pm$ 0.7			
FI	5.1 $\pm$ 0.6	19.4 $\pm$ 0.4			

*Average number of microorganisms (*Staphylococcus aureus*, opsonised with 1% human γ -globulin) phagocytised per granulocyte, $\pm$ s.e.

All tests on patients and on normal controls that were done on the same day are displayed horizontally. The differences in phagocytic activities between patients and normal controls are all significant to $P < 0.05$, except for LB ($P = 0.11$). The differences between contact angles measured on patients' and normal controls' granulocytes are all significant to $P < 0.05$.

to be present in the lesions of IJP. Rats monoinfected with these organisms rapidly develop severe alveolar bone loss, so these bacteria may be potential pathogens. They may release factors that penetrate the ulcerated gingival epithelium and enter the systemic circulation. Such factors may affect PMNL, resulting in altered functions such as chemotaxis and phagocytosis. Studies are under way to test this hypothesis. Alternatively, pre-existing impairment in PMNL function may exist in persons with IJP, and proliferation of these unique organisms may result from this diminished host response.

We have demonstrated that a reduction in PMNL phagocytosis and chemotaxis is common to patients with IJP. Whether this reduction in host protective response is pre-existing or a result of the disease remains to be determined.

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Allotypes of mouse IgM immunoglobulin

GENETIC polymorphism of the structural genes encoding the class-specific (heavy) polypeptide chains of the immunoglobulin (Ig) molecules provides a useful set of markers for elucidating the arrangement and expression of these genes. On the basis of various antigenic, physiochemical and biological properties, the immunoglobulins of the mouse have been divided into eight distinct classes, IgM, IgD, IgA, IgG₁, IgG_{2a}, IgG_{2b}, IgG₃ and IgE, with a specific structural gene determining the heavy chain (H) for each class. Previous studies have documented the existence of a genetic polymorphism for five of these H-chain genes¹⁻³, with all the genes being closely linked constituting a heavy chain chromosome region. The most recent of these loci to be demonstrated (Ig-5, determining the δ chain)³ was detected by the reaction of alloantisera prepared against spleen cells from mice differing in both *H-2* and allotype. These sera reacted with B lymphocytes of the appropriate allotype congenic strains, as assessed by either immunofluorescence or cell surface iodination and polyacrylamide gel electrophoresis (PAGE). As most B lymphocytes express cell surface IgM as well as IgD (refs 4-6), we have further pursued these various approaches to identify Ig allotypes and have found a polymorphism of the heavy chain (μ) of murine IgM, a molecule found in pentameric form (19S) in serum, and in monomeric (8S) form on the surface of lymphocytes. This defines a locus, Ig-6, which encodes the μ chain of most mouse IgM immunoglobulin.

Alloantisera containing anti-IgM antibodies were raised in various strain combinations by two different methods. A small proportion of anti-allotype sera raised by repeated immunisation with pertussis anti-pertussis complexes¹, were found to contain antibodies to allotypic determinants on IgM molecules. However, the most effective antisera against IgM allotype have been raised by spleen cell immunisation (4-6 weekly injections of 10⁷ spleen cells, with or without adjuvants) between strain combinations which differ in *H-2* type as well as allotype.

The binding capacities of these antisera against purified mouse immunoglobulins were assessed by radioimmunoassay¹ using purified ¹²⁵I-labelled mouse myeloma proteins². Each labelled myeloma protein was more than 80% precipitable by the appropriate heterologous anti-mouse heavy-chain serum, while less than 10% was precipitated by all other anti-H-chain sera. The IgM proteins used were HPC-76 and MOPC104 (IG-6a) of BALB/c origin, and C.BPC112 (IG-6b) of C.B/20 (BALB/c Ig^b) origin (kindly provided by Dr M. Potter). The allelic designations for Ig-6

Table 1 Anti-Ig-6 antibody detected by radioimmunoassay, immunofluorescence and cell surface radioiodination/polyacrylamide gel electrophoresis (PAGE)

Alloantiserum	Precipitation of ^{125}I IgM†		Positive immunofluorescence‡		PAGE analysis	
	HPC-76 (Ig-6a)	C.BPC112 (Ig-6b)	Strain	Splenic B cells	Strain	Heavy chain detected
GA20: C57BL anti-CBA* serum	Yes	No	BALB/c	Yes§	BALB/c	μ
GA26: C57 anti-CBA serum	No	No	BALB/c.Ig ^b	No§	BALB/c	—
MA3: C57BL anti-CBA spleen	Yes	No	C57.Ig ^a	Yes	CBA	μ, δ
			C57BL	No		
MB1: SJA anti-BALB/c.Ig ^b spleen	No	Yes	SJL	Yes	C57BL	μ, δ
			SJA	No		
MB2: SJA anti-BALB/c.Ig ^b spleen	No	No	SJL	Yes	C57BL	δ
			SJA	No		
MB3: SJA anti-CWB spleen	No	Yes	SJL	Yes	C57BL	μ
			SJA	No		

*CBA anti-pertussis serum/pertussis complex.

†Determined by radioimmunoassay as previously described¹.

‡Revealed by indirect immunofluorescence using a fluoresceinated goat anti-mouse IgG (refs 3 and 9).

§Analysis was by visual fluorescence microscopy and/or with a fluorescence-activated cell sorter (FACS).

Yes: almost all B cells were stained. No: few, if any, B cells were stained.

PAGE analysis of ^{125}I -labelled membrane protein precipitated by antisera³.

Mouse strains used in these experiments are of the following allotypes: Ig^a-CBA, BALB/c, SJA, C57.Ig^a; Ig^b-C57BL/6, BAB/c.Ig^b (BAB/14 or C.B/20), SJL, CWB (C3H.SW.Ig^b).

are assigned as for other allotype loci according to the Ig-1 allele of the type strains in which they are found². Thus, Ig-6a is from BALB/c and Ig-6b is from C57BL/6. The precipitating activity of such antisera against ^{125}I -labelled HPC-76 is shown in Fig. 1. Antisera GA20 and GA26 were both made in C57BL/6 (C57) against pertussis anti-pertussis (of CBA origin) complexes. Each contained anti-Ig-1a antibodies (Fig. 1b), but serum GA20 also contained anti-Ig-6a antibody (Fig. 1a). In contrast, the serum MA3 prepared in C57 mice against CBA spleen cells, contained no anti-Ig-1a antibody, but did contain anti-Ig-6a antibody (Fig. 1a and b).

The anti Ig-6a sera were specific since they did not precipitate an IgM myeloma of C.B/20 (Ig^b) origin, C.BPC112 (see Table 1). Their specificity was confirmed by inhibition studies in which no myeloma protein of other classes (IgA, IgG₁, IgG₂) inhibited precipitation of ^{125}I -labelled HPC-76. Furthermore, unlabelled HPC-76, MOPC104 and normal serum from BALB/c did inhibit this precipitation, although MOPC104 was considerably less effective in inhibiting than the other inhibitors.

Antisera to another IgM allotype, Ig-6a, were prepared in SJA (Ig^a) against spleen cells of Ig-1b positive strains BALB/c.Ig^b (BAB/14) or CWB (C3H.SW.Ig^b). These sera precipitated C.BPC112 but precipitated neither HPC-76 nor MOPC104 (Table 1). Inhibitions of precipitation were found

with Ig-6b containing sera and not with Ig-6a containing sera or myelomas. Similarly, no non-IgM myeloma protein inhibited ^{125}I -C.BPC112 precipitation with these alloantisera (data not presented).

Close linkage of Ig-6 to the Ig heavy chain region is implied in the use of allotype congenic strains for anti-serum testing. Anti-Ig-6a (for example, antiserum MA-3 in Fig. 1c) is inhibited in its precipitation of HPC-76 by serum from BALB/c (Ig^a) and not by serum from the congenic mice BALB/c.Ig^b (BAB/14) (Fig. 1c) nor from C.B/20 (data not shown) which have all the alleles of BALB/c except these closely linked to Ig-1. Since something more than 21 backcross generations are involved in the derivation of these strains (they are not completely independently derived), very close linkage is indicated for Ig-6 to Ig-1 and the other heavy-chain loci³.

These results with radioimmunoassay analysis demonstrate that antisera prepared against either cell-surface 8S IgM or serum 19S IgM, are capable of binding to 19S IgM molecules. We have also shown by immunofluorescence, that these antisera can react with cell-surface 8S IgM. Spleen cells from 8-week-old BALB/c (Ig^a), BALB/c.Ig^b, SJL (Ig^b), or SJL.Ig^a (SJA) allotype congenic strains were treated with a 5–10% (v/v) concentration of the alloantisera, washed and then reacted with a fluorescein-conjugated goat anti-mouse γ_1 and γ_2 antiserum. The latter serum was

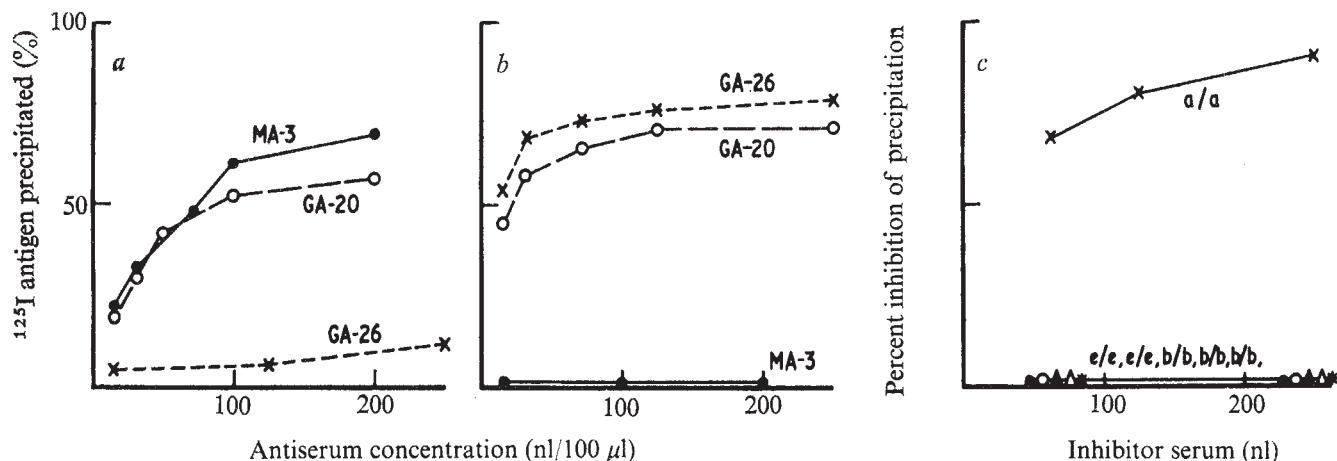


Fig. 1 Radioimmunoassay analysis of anti-Ig-6a antisera. The ability of alloantisera GA20, GA26 and MA3 to precipitate ^{125}I -labelled IgM (HPC-76 macroglobulin) or IgG_{2a} (HPC-149) of BALB/c origin is shown in a and b, respectively, as the per cent precipitation of the labelled antigen against the amount of antiserum used. For panel c cell assays were performed in a volume of 100 μl with 5 ng of ^{125}I -labelled HPC-76 (Ig-6a). All inhibitor sera derived from 8-week-old mice of the following allotype congenic strains: BALB/c (Ig^a), BALB/c.Ig^b; C57BL(Ig^b), C57BL.Ig^a NZB (Ig^c), NZB.Ig^b. Note that the lack of inhibition by Ig^c sera indicates that Ig-6^a and Ig-6^c do not cross react with this antiserum. Further details of antigenic specificity will be published.

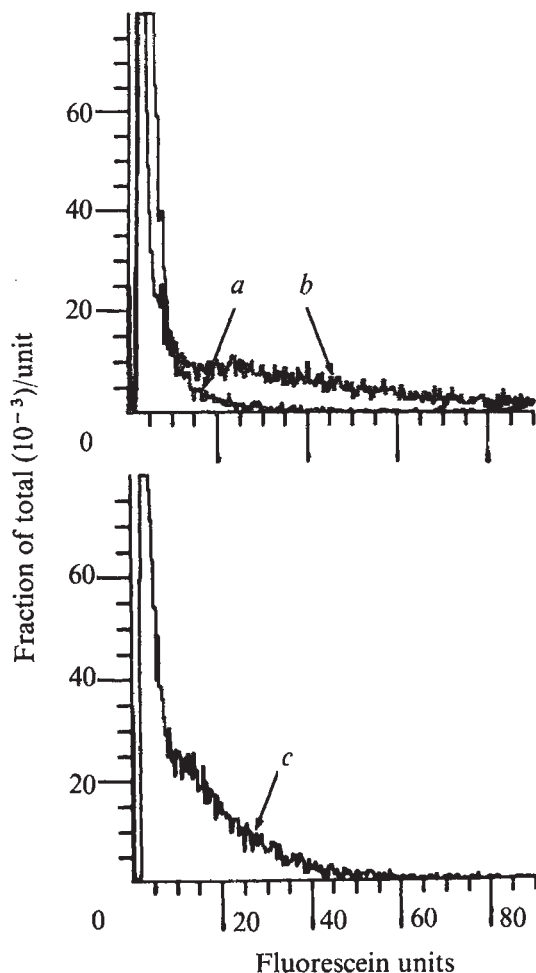


Fig. 2 FACS fluorescence analysis of anti-Ig-6b serum. Fluorescence profile of SJA(Ig^a) anti-BALB/c.Ig^b serum on (a) SJA spleen cells (Ig^a), (b) SJL spleen cells (Ig^b), (c) SJL spleen cells after adsorption of serum with Sepharose-bound IgM myeloma C.BPC112 (Ig-6b). Cells (2×10^6) were reacted with 2 μ l (diluted to 40 μ l with buffered saline containing 0.2% sodium azide) SJA anti-BALB/c.Ig^b serum for 20 min at room temperature. These cells were washed and stained with fluoresceinated goat anti-mouse IgG for a further 20 min at room temperature and analysed on the FACS. Adsorption of this anti-Ig-6b serum by Sepharose-bound Ig-6a did not reduce its staining activity for SJL spleen cells.

completely non-reactive to IgM, as shown by immunofluorescence and radioimmunoassay. Fluorescence was detected by fluorescence microscopy as previously described³, or by flow fluorometry with a fluorescence-activated cell sorter (FACS)⁸. These data are summarised in Table 1.

Table 1 shows that the same sera which precipitate secreted IgM also bind to cell surface IgM. Antisera made against pertussis-anti-pertussis complexes (that is, against secreted IgM) could be tested on spleen cells of any strain whereas antisera made against spleen cells could not because of the possible presence of antibodies to other cell surface alloantigens. With these latter antisera, spleen cells from mice congenic but for allotype with the antibody produced were used.

The antisera made in C57(Ig^b) against CBA(Ig^a) stained C57.Ig^a spleen cells and, of course, did not stain C57 cells (Table 1). Similarly, antisera made in SJA(Ig^a) against Ig^b cells stained SJL(Ig^b) but not SJA cells (Table 1 and Fig 2, a and b). Thus the surface molecules detected by these antisera are coded by genes in the Ig heavy-chain chromosome region.

The immunoglobulin class of the cell-surface molecules detected in these immunofluorescent reactions was determined by adsorption and blocking studies as well as by gel

electrophoresis (PAGE). Serum MB1, SJA anti-BALB/c.Ig^b spleen, passed through a column of Sepharose-conjugated C.BPC112, an IgM of b allotype (Ig-6b), lost most of its staining activity for SJL (Fig. 2c). Therefore most of the reactivity is directed towards IgM. The antithetical serum against Ig-6a was similarly adsorbed by Sepharose MOPC104, an IgM myeloma from the Ig^a strain BALB/c (data not shown). Neither conjugate removed any staining activity from the opposite antisera. Heterologous anti-mouse μ chain blocked staining by both these antisera as well as by serum GA20. These same allotype antisera also precipitate cell surface IgM shown by PAGE of radioiodinated membrane proteins^{3,7}. Thus, these alloantisera detect the allotypes Ig-6a and Ig-6b on cell-surface IgM.

Some antisera prepared by allogeneic spleen cell injections also have antibodies against IgD heavy-chain allotypes (Ig-5)^{3,9}. Serum MB2 stained SJL spleen cells, although it contained no anti-Ig-6b reactivity as shown by radioimmune precipitation. This staining activity of SJL cells by serum MB2 was not removed by adsorption with insolubilised C.BPC112 nor was it blocked by pretreatment with heterologous anti-mouse μ . MB2 (Table 1) also precipitates radioiodinated cell surface IgD demonstrated by PAGE (ref. 3). Anti-Ig-5b activity also accounts for the small residual staining of IgM-adsorbed serum MB1 (Fig. 2c). The μ and δ precipitation activity of each of the sera detected by PAGE is given in the last column of Table 1. These data show that sera made against allogeneic spleen cells may have anti-allotype antibodies to δ chains (Ig-5), μ chains (Ig-6) or both in varying amounts. These new allotypes will serve as useful markers in following B-cell differentiation and gene expression. They may also aid in fine structure mapping of the Ig heavy-chain region which contains genes for both the constant and variable parts of the polypeptides. Some of these data has been presented⁹. A more detailed immunogenetic analysis of these loci will be published elsewhere.

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Specific glucocorticoid inhibition of growth promoting effects of prostaglandin $F_{2\alpha}$ on 3T3 cells

GROWTH and differentiation of mammalian cells is considered to be controlled in part by the interaction of various hormones and other growth promoting factors¹. Corticosteroids are hormones with a wide range of tissue specificity, intracellular receptor proteins having been described in various tissues² and cultured cells^{3,4}. Glucocorticoids exert paradoxical effects on cell proliferation. Thus they promote cell growth of the mammary gland epithelium in mice and rats^{5,6} and in mouse and rat organ⁷⁻⁹ and rat tissue culture systems¹⁰, and DNA synthesis in cultured parenchymal rat liver cells¹¹. But they inhibit all stages of the inflammatory response, including the reparative growth of fibroblasts, loss of immune competence due to damage to lymphocytes, lipolysis and other anabolic (contra-insulin) effects in adipose tissue¹². In cultured fibroblasts hydrocortisone can, in different conditions, either stimulate¹³ or inhibit¹⁴⁻¹⁶ DNA synthesis and cell division. The growth promoting effects of the corticosteroids in fibroblasts and mammary epithelial cells may be related to their ability to synergise with the fibroblast growth factor (FGF) (refs 17 and 18) and prolactin¹⁰ respectively. Growth inhibitory effects may be due to the predominantly catabolic action of the glucocorticoids¹². Here we report that hydrocortisone can inhibit specifically the stimulation of DNA synthesis and cell division induced by prostaglandin $F_{2\alpha}$ (ref. 19) in Swiss mouse 3T3 and 3T6 cells. We suggest that the cell-growth-regulating properties of the corticosteroids are in part dependent on the identity of the co-inducing growth controlling factors, and that glucocorticoid inhibition of prostaglandin-stimulated cell growth may have a role in the anti-inflammatory response.

Prostaglandin $F_{2\alpha}$ added at 500 ng ml⁻¹ to relatively quiescent 3T3 cells stimulated 20% of the cells to synthesise DNA in 26 h, whereas without any additions only 0.3% of the cells were synthesising DNA (Fig. 1). Addition of increasing concentrations of hydrocortisone with $PGF_{2\alpha}$ caused a reduction in the fraction of cells synthesising DNA, which reached a maximum of 85% inhibition at a concentration of 20 ng ml⁻¹ (Fig. 1 insert). Addition of insulin (50 ng ml⁻¹) alone had little effect, but when added with $PGF_{2\alpha}$ it increased the fraction of cells synthesising DNA, thus showing a synergistic effect. The addition of hydrocortisone to $PGF_{2\alpha}$ and insulin, however, caused only partial suppression of the increase in DNA synthesis measured in the same period (40% inhibition with hydrocortisone at 40 ng ml⁻¹), even at high glucocorticoid concentrations (Fig. 1). Also the concentration of hydrocortisone required to produce half maximal inhibition of DNA synthesis was increased from 1 to 4 × 10⁻⁸ M in the presence of insulin (Fig. 1 insert). Addition of hydrocortisone alone or with insulin caused no stimulatory effect (Fig. 1). Preincubation of the quiescent cultures for 24 h with hydrocortisone (40 ng ml⁻¹) did not prevent the cells (95%) from synthesising DNA after addition of 10% serum.

The inhibitory effect of hydrocortisone on the stimulation of DNA synthesis produced by $PGF_{2\alpha}$ or $PGF_{2\alpha}$ and insulin was relatively specific for glucocorticoids. Thus increasing concentrations of dexamethasone as well as hydrocortisone inhibited the $PGF_{2\alpha}$ -stimulation, whereas oestradiol, cortisone and testosterone were without effect (Fig. 2). Progesterone with or without insulin respectively caused only a marginal decrease or increase in the stimulatory effect of $PGF_{2\alpha}$. The concentration of dexamethasone required to cause 50% of the final reduction in DNA synthesis with $PGF_{2\alpha}$ was approximately ten times less than the corresponding hydrocortisone concentration. This correlates well with the difference in their relative glucocorticoid activities²⁰. The failure of cortisone to inhibit $PGF_{2\alpha}$ -stimulated DNA synthesis may be explained in part by the failure of 3T3 cells to convert cortisone into its active form,

hydrocortisone²⁰. Addition of hydrocortisone (400 ng ml⁻¹) to quiescent 3T6 cells plated in the absence of serum also caused a reduction in the fraction of cells synthesising DNA after a 24-h exposure to $PGF_{2\alpha}$ and insulin (Table 1). Thus the inhibitory effect of hydrocortisone was not due to the steroid interacting with other agents or hormones present in serum.

The capacity of hydrocortisone to inhibit DNA synthesis induced by $PGF_{2\alpha}$ was also reflected in its inhibition of cell multiplication. Hydrocortisone inhibited the increase in 3T3 cell numbers induced by $PGF_{2\alpha}$ or $PGF_{2\alpha}$ plus insulin after 48 h when added to quiescent cultures. Similar results were obtained when hydrocortisone was added to rapidly proliferating cells (not shown). Oestradiol at the same concentrations had no effect (Fig. 2a). That $PGF_{2\alpha}$ was acting directly on the cells and not through any intermediary metabolites and that hydrocortisone directly antagonised its action was suggested as follows. (1) Indomethacin (5 μM), which blocks the synthesis

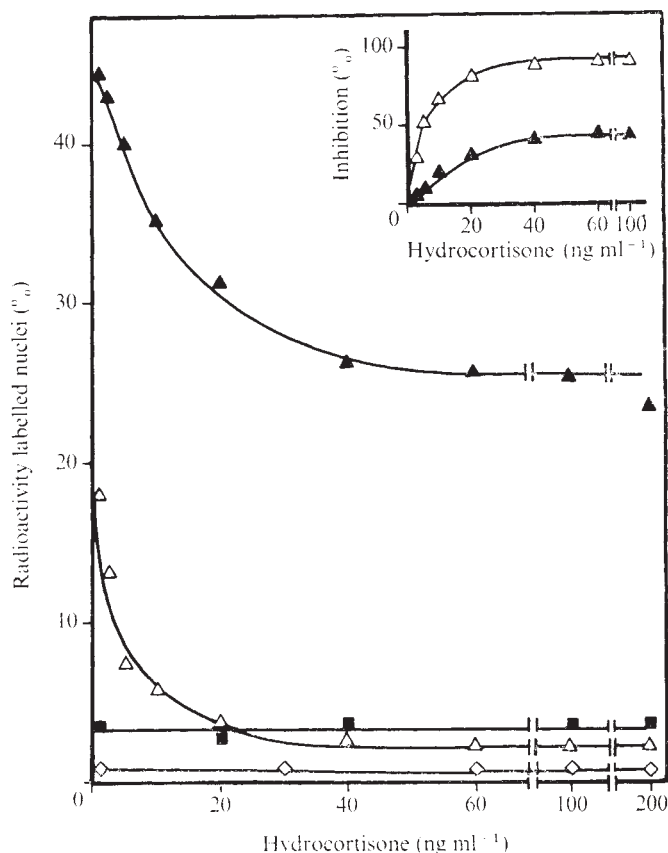


Fig. 1 Effect of various concentrations of hydrocortisone on the stimulation of DNA synthesis by $PGF_{2\alpha}$ and insulin. Additions to the cultures were as follows: $PGF_{2\alpha}$, 500 ng ml⁻¹ (△); insulin, 50 ng ml⁻¹ (■); $PGF_{2\alpha}$ and insulin (▲) and a control of no additions (◇) for increasing concentrations of hydrocortisone. The insert shows the percentage inhibition of the stimulation of DNA synthesis produced by hydrocortisone with either $PGF_{2\alpha}$ (△) or $PGF_{2\alpha}$ and insulin (▲). Swiss mouse 3T3 fibroblasts³¹ were maintained in Dulbecco's modified Eagle's medium (DEM) containing penicillin (100 U ml⁻¹), streptomycin (100 μg ml⁻¹) and 10% foetal calf serum in 90-mm Nunc Petri dishes as described before¹⁹. For the determination of radioactively labelled cell nuclei, the cells were plated at 10⁵ per 33-mm dish in 2 ml of DEM supplemented with 0.03 μM biotin, 0.05 μM vitamin B₁₂, 140 μM aspartic acid, 0.3 mM glutamic acid, 50 μM glutathione, 60 μM hypoxanthine and 5% foetal calf serum. The presence of the additional low molecular weight nutrients increased the fraction of cells synthesising DNA in the presence of $PGF_{2\alpha}$ alone with insulin when compared with those values reported¹⁹ without supplementation³² (M. K. O., D. C., P. S. R. and L. J. de A. in preparation). Methyl-³H-thymidine was added to the medium at 3 μCi ml⁻¹, 1 μM from 0 until 26 h after the additions and the cells were continuously exposed to the extra additions until the cultures were processed for autoradiography as described before^{18,19}.

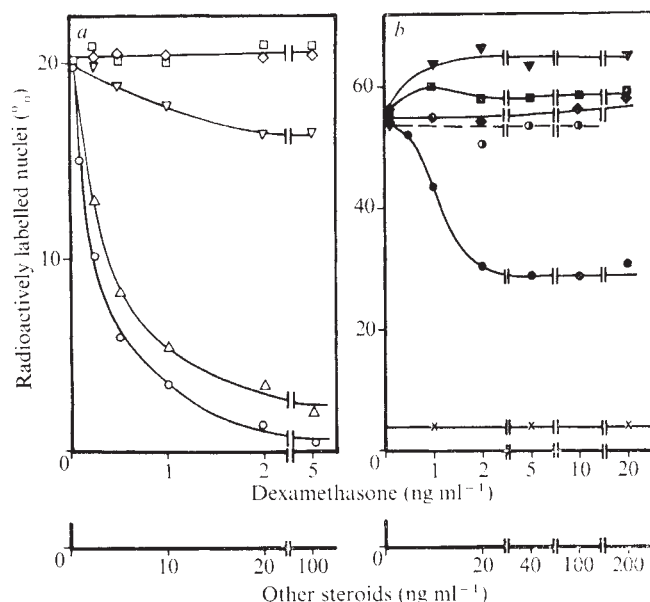


Fig. 2 Effect of various steroids on the stimulation of DNA synthesis induced by $\text{PGF}_{2\alpha}$ and insulin. *a*, Increasing concentrations of dexamethasone (○) (upper scale), hydrocortisone (△), cortisone (□), testosterone (◇) and progesterone (▽) (all on lower scale) were added to 3T3 cultures containing $\text{PGF}_{2\alpha}$ (500 ng ml^{-1}). *b*, Increasing concentrations of dexamethasone (●) (upper scale), cortisone (■), testosterone (◆), progesterone (▼), and oestradiol (⬤) (all on lower scale) were added, together with $\text{PGF}_{2\alpha}$ and insulin (50 ng ml^{-1}). A control with insulin and dexamethasone alone is shown (×). The cells were radioactively labelled for autoradiography from 0 until 26 h after additions as described in Fig. 1.

of prostaglandins from their precursors in canine spleen²¹, failed to inhibit the $\text{PGF}_{2\alpha}$ -stimulated increase in DNA synthesis with or without insulin, while polyphlorethyl phosphate and SC 19220, inhibitors of the action of prostaglandins²², also inhibited the mitogenic effect of $\text{PGF}_{2\alpha}$ in 3T3 cells (Table 1). (2) Stable analogues of the intermediary endoperoxides G_2 and H_2 , which have similar physiological effects to $\text{PGF}_{2\alpha}$ in bronchoconstriction in animals²³ failed to stimulate cell proliferation with or without insulin present. (3) Possible antimitogenic prostaglandins which might have been synthesised or released after addition of hydrocortisone, such as PGE_1 and

Table 2 Effect of hydrocortisone on the stimulation of DNA synthesis by prostaglandin $\text{F}_{2\alpha}$ and insulin in 3T6 cells

Additions	DNA synthesis (% labelled nuclei)
None	1.3
$\text{PGF}_{2\alpha}$	14.0
Insulin	7.0
$\text{PGF}_{2\alpha}$ + insulin	31.0
$\text{PGF}_{2\alpha}$ + hydrocortisone	2.8
$\text{PGF}_{2\alpha}$ + insulin + hydrocortisone	12.4
Serum	100.0

Stock plates of Swiss mouse 3T6 cells³¹ were grown in DEM with 10% foetal calf serum in 90-cm Petri dishes until the cultures were nearly confluent. The medium was removed, the cell monolayers were rinsed with 5 ml phosphate-buffered saline (0.17 M NaCl; 0.005 M KCl; 0.01 M sodium phosphate, pH 7.0), and the cultures were digested with 2 ml PBSA containing trypsin (100 $\mu\text{g ml}^{-1}$) for 1 min at 37 °C. The trypsin solution was then removed, and the cells were resuspended in 2.5 ml of DEM containing 0.1% serum and soybean trypsin inhibitor (200 $\mu\text{g ml}^{-1}$, Sigma, T 9003) and collected by centrifugation³². Cells were resuspended in 5 ml DEM and 0.1% serum, diluted 40-fold into DEM containing the extra nutrients described in Fig. 1, and then plated in 2 ml of medium at 1.5×10^5 cells per 33 mm dish. Final serum concentration was 0.0025%. Additions of PGF (500 ng ml^{-1}), insulin (50 ng ml^{-1}), hydrocortisone (40 ng ml^{-1}) and serum 10% were made 4 d later as indicated. The fraction of cells synthesising DNA 24 h later was recorded as described in Fig. 1.

PGE_2 at low concentrations, failed to inhibit the mitogenic effect of $\text{PGF}_{2\alpha}$ or $\text{PGF}_{2\alpha}$ plus insulin in 3T3 cells (unpublished results).

The mechanism of action of the glucocorticoid-induced inhibition of DNA synthesis and its partial reversal by insulin is unknown, although glucocorticoid-induced reductions in glucose²⁴ rubidium and phosphate²⁵ uptakes have been reported in different cells and these transport systems are stimulated by $\text{PGF}_{2\alpha}$ or insulin in mouse fibroblasts²⁶. Transport alterations probably do not mediate directly the inhibitory effect of glucocorticoids, since their reduction of the uptake of rubidium ions and phosphate is dependent on protein synthesis in rat thymocytes²⁵. Furthermore, the growth of mouse L929 cells in medium containing high concentrations of fructose instead of glucose where the sugar enters the cell by passive diffusion rather than by carrier mediated transports²⁴ can also be inhibited by glucocorticoids. It is surprising that hydrocortisone, a predominantly catabolic hormone¹² can increase cell proliferation with FGF in fibroblastic cells^{17,18}, or with prolactin in mammary epithelial cultures¹⁰. Hydrocortisone, however, may

Table 1 Effect of inhibitors and analogues of $\text{PGF}_{2\alpha}$ on DNA synthesis and cell division

Additions	DNA synthesis (% ^3H -labelled nuclei)	Cell no. ($\times 10^{-5}$)
None (control)	0.2	6.03 ± 0.22
$\text{PGF}_{2\alpha}$	18.0	7.57 ± 0.03
$\text{PGF}_{2\alpha}$ + hydrocortisone	1.7	6.28 ± 0.22
$\text{PGF}_{2\alpha}$ + insulin	41.0	14.81 ± 1.1
$\text{PGF}_{2\alpha}$ + insulin + hydrocortisone	23.0	9.19 ± 0.4
$\text{PGF}_{2\alpha}$ + PPP	1.2	6.02 ± 0.0
$\text{PGF}_{2\alpha}$ + insulin + PPP	22.0	7.87 ± 0.6
$\text{PGF}_{2\alpha}$ + SC-19220	5.2	6.89 ± 0.6
$\text{PGF}_{2\alpha}$ + indomethacin (2.5 μM)	19.0	—
$\text{PGF}_{2\alpha}$ + indomethacin (5.0 μM)	17.7	8.85 ± 1.0
$\text{PGF}_{2\alpha}$ + insulin + indomethacin (2.5 μM)	42.0	—
$\text{PGF}_{2\alpha}$ + insulin + indomethacin (5.0 μM)	39.0	12.8 ± 0.3
Insulin	3.9	6.5 ± 0.6
U-44069	0.2	5.6 ± 0.2
U-46619	0.2	6.3 ± 0.1
Serum	97	24.50 ± 1.2
Serum + hydrocortisone	97	23.9 ± 0.2

3T3 cultures were plated at 10^5 cells per 33-mm Petri dish for the determination of DNA synthesis or 1.5×10^5 cells in 5 ml of medium per 5-cm Petri dish for determination of cell numbers as described in Fig. 1. After 4 d, when the cultures became stationary, $\text{PGF}_{2\alpha}$ (500 ng ml^{-1}); hydrocortisone (40 ng ml^{-1}), insulin (50 ng ml^{-1}); polyphlorethyl phosphate²² (PPP, 5 $\mu\text{g ml}^{-1}$); 1-acetyl-2-(8-chloro-10,11-dihydrodibenz(b,f)(1,4)oxazepine-10-carbonyl)hydrazine²² (SC 19220) (10 $\mu\text{g ml}^{-1}$); indomethacin 2.5 or 5 μM ; (15S)-hydroxy-9 α ,11 α (epoxymethano)prosta-5z,13-dienoic acid²³ (U-44069, 500 ng ml^{-1}); (15S)-hydroxy-11 α ,9 α (epoxymethano)prosta-5z,13-dienoic acid²³ (U-46619, 500 ng ml^{-1}), and 10% foetal calf serum were added as indicated. The fraction of cells synthesising DNA by 26 h or the numbers of cells $\pm$ s.e. observed by 48 h after additions is shown. In controls the amount (μg) of DNA isolated³³ per 10^5 cells 48 h after the addition of: nothing; $\text{PGF}_{2\alpha}$; $\text{PGF}_{2\alpha}$ + hydrocortisone; $\text{PGF}_{2\alpha}$ + insulin; $\text{PGF}_{2\alpha}$ + insulin + hydrocortisone; $\text{PGF}_{2\alpha}$ + insulin + indomethacin; serum was 11.6 ± 0.4 ; 15.0 ± 0.4 ; 12.2 ± 1.1 ; 19.3 ± 0.3 ; 14.1 ± 1.5 ; 19.2 ± 0.4 and 18.7 ± 2.1 respectively.

increase the efficiency of pituitary polypeptide interaction with the membrane in a manner similar to the way in which it specifically enhances lipolytic action of pituitary ACTH in fat cells²⁷.

In conclusion, both positive and negative effects of corticosteroids on the growth of cultured mouse fibroblasts have been reported. The same concentrations of hydrocortisone can either increase the rate of DNA synthesis induced by FGF (refs 17, 18) or decrease the rate induced by PGF_{2α} in the same Swiss mouse 3T3 cell cultures. Thus the cell growth regulatory properties of the glucocorticoids can be determined by the identity of the co-inducing growth controlling agents: this may in part explain their paradoxical effects in tissue culture. The exact physiological relevance of these observations is unknown. *In vivo* corticosteroids are potent anti-inflammatory agents²⁸, while prostaglandins may mediate some of the effects of the inflammatory process²¹. Hydrocortisone can inhibit both PGF_{2α}-stimulated cell growth and collagen production in fibroblasts^{28,29}, so corticosteroid anti-inflammatory activity against prostaglandins may involve direct competition for control of various cellular processes including cell multiplication, and may not be solely due to inhibition of the production of prostaglandins³⁰.

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Altered cyclic AMP-binding and db cyclic AMP-unresponsiveness *in vivo*

Cyclic AMP has been implicated in the regulation of cell growth. However, the molecular mechanism of cyclic AMP action, especially that involving the control of tumour growth *in vivo* is not clear. Our previous studies on two cell populations of Walker 256 mammary carcinoma (W256), one regressing (responsive), the other growing (unresponsive) under dibutyryl (db) cyclic AMP treatment, showed a correlation between altered cyclic AMP-binding of the tumour cytosol and db cyclic AMP-unresponsiveness¹. Deficient cyclic AMP-binding in other malignant growth has also been reported²⁻⁴. It is suggested that the defective cyclic AMP-binding proteins may be the possible cause for

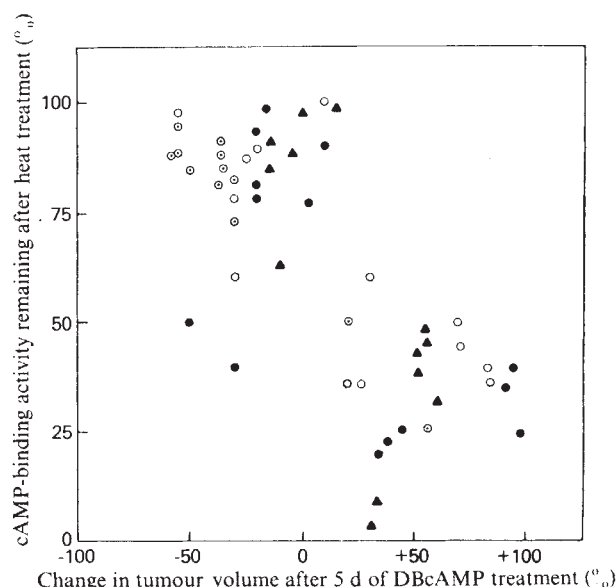


Fig. 1 Correlation between heat stability of cyclic AMP-binding and db cyclic AMP-induced tumour regression. ○, MTW9; ●, NMU; ○, R3230AC; ▲, hepatoma 5123. Growth of tumours was measured daily and expressed as the percentage change in volume⁹. Rats bearing 2-3-g tumours were injected⁹ with db cyclic AMP for 5 d and killed 5 d later. Tumours were homogenised with 5 volumes of Tris-HCl buffer, 10 mM (pH 7.5). Protein concentrations of the cytosols (105,000g, 1 h) were adjusted to 2 mg ml⁻¹ with the buffer. Aliquots of the cytosols were placed in glass tubes and heated (in duplicate) in a 50 °C water bath for 15 min stirring gently with a glass rod, then cooled to 0 °C. The unheated and heated cytosols were assayed for cyclic AMP-binding at 0 °C for 90 min by Gilman's competition protein binding assay⁶ as previously described¹. High affinity binding was determined at 10⁻⁸ M cyclic AMP (Y.S.C.-C. *et al.*, in preparation). Tumour cytosols were also heated as above after pre-incubation of cytosols with non-radioactive cyclic AMP (10⁻⁷ M) at 0 °C for 90 min. Both heated and unheated cytosols which were presaturated with cold cyclic AMP, were then treated with Dextran coated charcoal (DCC) to remove unbound cyclic AMP (Y.S.C.-C. and T.C., in preparation). One ml (2 mg protein) of each cytosol was added to a 400g DCC pellet derived from 4.0 ml of the DCC suspension (0.25% Norit A and 0.0025% Dextran, Grade C, in 0.01 M Tris-HCl, pH 8.0); the mixtures were incubated in a 23 °C water bath for 15 min with intermittent shaking every 5 min, then cooled to 0 °C, and centrifuged. The supernatants were assayed for cyclic AMP-binding at 2 × 10⁻⁸ M cyclic AMP by the modified method of Do Khac *et al.*⁷ to enhance the cyclic AMP exchange. The reaction was carried out at 23 °C for 3 h. The specific binding activity⁸ was expressed as the percent activity of the unheated control. Each symbol represents the average of duplicate determinations of a single tumour. Tumours frozen at -70 °C for up to 2 weeks gave results similar to those on unfrozen material. The heat stability test was also done with tumour biopsy specimens before db cyclic AMP treatment. The same correlation between the stability of the binding proteins and growth arrest was obtained. Protein concentrations were determined by the method of Lowry *et al.*⁸

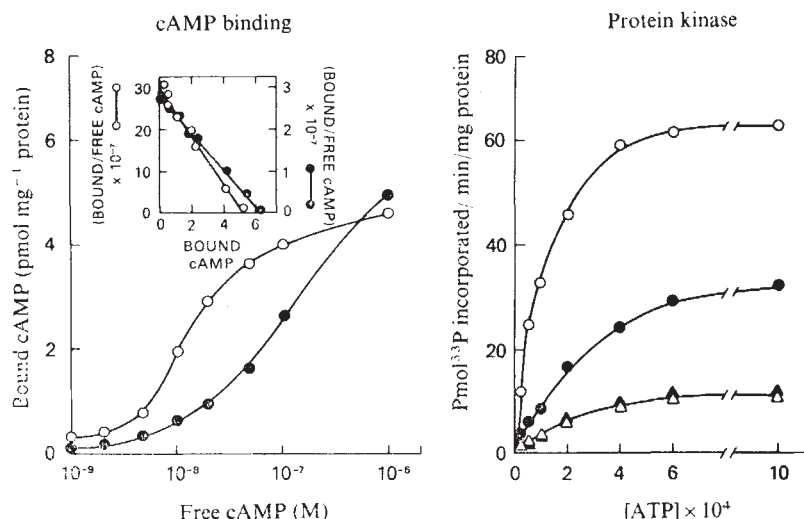
the loss of growth control by cyclic AMP. To determine the relationship between the qualitative difference of cyclic AMP-binding proteins and db cyclic AMP-responsiveness *in vivo*, we have used various experimental tumour models, such as, transplantable mammary carcinoma (MTW9) in Wistar-Furth inbred female rats, a hormone-dependent tumour; primary, *N*-nitrosomethylurea-induced mammary carcinoma (NMU) and transplantable mammary adenocarcinoma (R3230AC) in Fischer female rats, hormone-responsive tumours; and hepatoma 5123 in Buffalo/N female rats.

Figure 1 shows a relationship between heat stability of cyclic AMP-binding proteins in tumour cytosol and db cyclic AMP-responsiveness *in vivo* with 4 types of rat tumours. The high-affinity binding activity (Y.S.C.-C., *et al.*, in preparation) of the cytosols from unresponsive tumours (growing under db cyclic AMP treatment) was reduced to 50% or less when the cytosol was pre-incubated at 50 °C for 15 min; under the same treatment, more than 75% of the binding activity of the cytosols from responsive tumours (regressing under db cyclic AMP treatment) was retained. Mixing of cytosols from both responsive and unresponsive tumours gave additivity in the heat stability, suggesting that the heat lability of unresponsive tumour binding was not due to enzymatic degradation of the binding proteins. Presaturation of free binding sites with unlabelled cyclic AMP did not alter the stability of the binding proteins; this suggests that the heat stability of the binding activity of the responsive tumours was not due to high cyclic AMP concentrations in the tumour cytosols which might protect against the inactivation of the binding protein. Moreover, 8 mM β -mercaptoethanol decreased the heat lability of the binding in the unresponsive tumours but had little or no effect on the stability of the binding in the responsive tumours. Thus, there seems to be a difference in the structure or conformation of the binding proteins between the responsive and the unresponsive tumours. For 44 of 52 tumours there was a striking parallel between stable cytoplasmic cyclic AMP-binding proteins and db cyclic AMP-induced tumour regression *in vivo* (Fig. 1). A correlation between heat stability of cyclic AMP-binding proteins and db cyclic AMP-responsiveness has also been found in cultured neuroblastoma cells by Simantov and Sachs¹⁰ who reported that cells resistant to the cytotoxic effect of db cyclic AMP had cyclic AMP-binding proteins more sensitive to temperature than non-resistant cells.

The results in Fig. 2 show that the thermal lability of cyclic AMP-binding is associated with a reduced affinity of the binding proteins for cyclic AMP. The specific binding curves of db cyclic AMP-responsive and -unresponsive R3230AC tumours showed that the binding proteins in unresponsive tumour had a reduced apparent affinity for cyclic AMP, but the maximum binding sites in both tumours were similar. Scatchard analysis¹¹ of the binding curves gave K_d of 2.0×10^{-8} M and 1.2×10^{-7} M, for responsive and unresponsive tumours, respectively (Fig. 2, left, inset). It has been shown that cyclic AMP-binding proteins are a regulatory subunit of many mammalian protein kinases and the binding of cyclic AMP to the regulatory subunit stimulates the kinase activity¹³⁻¹⁹. Fig. 2 shows that cyclic AMP stimulation of protein kinase is apparently reduced in unresponsive tumour, whereas the K_m of the enzyme for ATP in the presence or absence of cyclic AMP is similar in responsive and unresponsive tumours (Fig. 2, right). These data are consistent with the difference in binding affinity for cyclic AMP observed between responsive and unresponsive tumours (Fig. 2, left).

The depression of cytosol binding activity of responsive W256 after db cyclic AMP treatment could be the result of an increase in endogenous binding of unlabelled cyclic AMP which decreases the exogenous ³H-cyclic AMP-binding². Alternatively, the decrease in the binding by the cytosol may reflect the change in intracellular compartmentalisation of binding protein and an actual loss of binding protein from the cytosol after db cyclic AMP treatment. These possibilities were examined by measuring cyclic AMP-binding and protein kinase activities in cytosols and nuclei from db cyclic AMP-treated and control tumours using sucrose gradient centrifugation (Fig. 3). Cytosol from the db cyclic AMP-responsive R3230AC control (a) exhibited two major peaks of cyclic AMP-binding activity, sedimenting at 4.3S and 5.6S, respectively. The cytosol also contained cyclic AMP-independent and -dependent forms of protein kinase, sedimenting at 3.3S and 5.6S, respectively. The latter sedimentation constant is identical to that of the heavier cyclic AMP-binding peak. Cytosol from the db cyclic AMP-unresponsive control tumour (e) showed a different sedimentation pattern of cyclic AMP-binding and protein kinase activities: the binding component sedimenting at 4.3S and the cyclic AMP-independent protein kinase sedimenting at 3.3S decreased while the higher molecular weight species of these proteins which were not apparent in the responsive tumour cytosol increased

Fig. 2 Cyclic AMP-binding and protein kinase activities of db cyclic AMP-responsive (open symbols) and -unresponsive (closed symbols) R3230AC tumours. ○, ●, Cyclic AMP binding or protein kinase activity in the presence of cyclic AMP; △, ▲, Protein kinase activity in the absence of cyclic AMP. Db cyclic AMP-responsive and -unresponsive tumours were obtained from standard R3230AC transplants following a method previously described¹². The preparation of tumour cytosol was the same as in legend to Fig. 1. Cyclic AMP binding was assayed at various concentrations of cyclic AMP as previously described¹. Protein kinase activity was determined by the measurement of ³²P incorporation from γ -labelled ATP into histone. The reaction mixture (0.25 ml) contained: 100 mM potassium phosphate buffer (pH 7.5); 20 mM magnesium acetate; 1 mM theophylline; 0.6 mg calf thymus histone; 3×10^{-7} M (γ -³²P) ATP, 19 Ci mmol⁻¹ (ICN Pharmaceuticals, Inc., Pikesville, Md.) with various concentrations of non-radioactive ATP; and cytosol (70 μ g protein). Incubations were carried out at 30 °C for 5 min $\pm$ 1 μ M cyclic AMP and the reactions were stopped by the addition of 0.5 ml of ice-cold 20% TCA. After standing for 30 min in an ice bath, the samples were filtered on to Millipore filters (0.45 μ m). The filters were then washed 6 times with 5 ml of 5% TCA, dried and counted by liquid scintillation. Values represent one of the 2 similar experiments and 5 pooled tumours were used in each experiment.



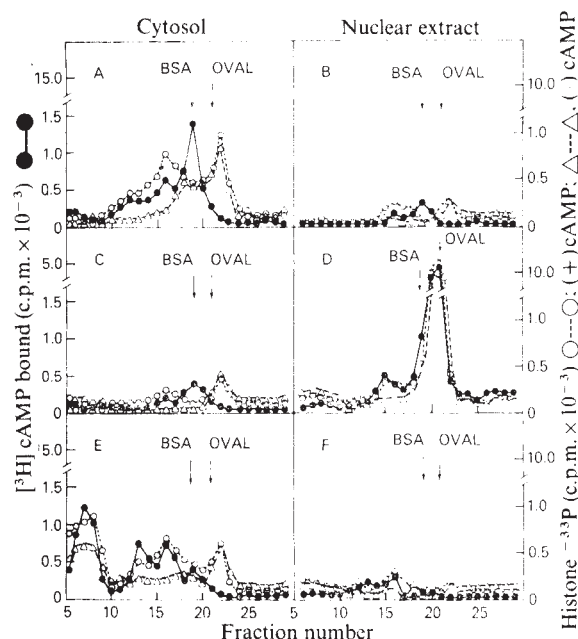


Fig. 3 Sucrose density gradient centrifugation of cytosols and nuclear extracts of db cyclic AMP-responsive and -unresponsive R3230AC mammary tumours before and after db cyclic AMP treatment. *a, b*, db cyclic AMP-responsive control; *c, d*, db cyclic AMP-responsive treated (db cyclic AMP treatment³ for 5 days); *e, f*, db cyclic AMP-unresponsive control (db cyclic AMP-unresponsive-treated was similar to *e* and *f*). See description of Fig. 2 for db cyclic AMP-responsive and -unresponsive tumours. Tumours were homogenised in a Teflon-glass homogeniser with 2 volumes of 10 mM Tris-HCl buffer (pH 7.5). The supernatants (105,000g for 1 h) were used as cytosols. The pellets from 105,000g were rehomogenised in an original volume of 20 mM Tris-HCl, 2 mM MgCl₂, 1 mM CaCl₂, pH 7.5 (buffer A). These homogenates were passed through 3 layers of gauze and centrifuged at 770g for 10 min. The pellets were resuspended and homogenised in the same volume of buffer A, then centrifuged at 770g for 10 min. The nuclear pellets thus obtained were homogenised in one volume of 0.4 M KCl in 10mM Tris-HCl buffer (pH 7.5) and extracted at 0°C for 90 min. The suspensions were centrifuged at 10,000g for 10 min and the clear supernatants used as nuclear extracts. Samples of cytosols and nuclear extracts containing 1.0 mg of protein were incubated at 23°C for 3 h, with 10⁻⁶ M [³H]-cyclic AMP, 10 mM theophylline and 50 mM potassium phosphate buffer (pH 7.0) then layered on 5–20% sucrose gradient in 10 mM Tris-HCl buffer (pH 7.0) (nuclear gradient included 0.4 M KCl). Centrifugation was at 2°C for 17 h at 45,000 r.p.m. in a Beckman SW 50.1 rotor. Thirty fractions were collected and assayed for cyclic AMP-binding (protein-bound ³H content) and for protein kinase activity. For the determination of cyclic AMP-binding, aliquots of each fraction were filtered onto Millipore filters (0.45 µm), washed and counted by liquid scintillation. Protein kinase activity was determined as in the legend to Fig. 2 at 0.5 mM ATP concentration. Sedimentation coefficients were determined by the method of Martin and Ames²⁰ using BSA (Bovine Serum Albumin, S₂₀, w = 4.5S) and OVAL (Ovalbumin, S₂₀, w = 3.6S) as internal standards. The values represent one of the 3 similar experiments.

markedly. The sedimentation profiles of cyclic AMP-binding and protein kinase of control nuclei from both responsive and unresponsive tumours (*b* and *f*) showed small broad peaks. In contrast, the nuclear extract from the db cyclic AMP-treated-responsive tumour (*d*) gave a sharp sedimentation profile composed mainly of a 3.6S species containing both cyclic AMP-binding and cyclic AMP-independent protein kinase activities. This increase in nuclear binding and kinase activities was accompanied by a decrease of these protein species in the cytoplasm (*c*).

The nuclear accumulation of cyclic AMP-binding and protein kinase components was not found in the db cyclic AMP-unresponsive tumour following the db cyclic AMP treatment; the sedimentation profiles of these macromolecular protein species in both the cytosol and nuclear extracts were the same as those in the untreated control

tumour. The differences observed between db cyclic AMP-treated-responsive and -unresponsive tumour nuclei with respect to cyclic AMP-binding and protein kinase activities was not due to the presence of a modulator of the binding and kinase in the extracts since mixing of db cyclic AMP-treated-responsive and -unresponsive tumour nuclear extracts exhibited additivity of the binding and kinase activities. A similar correlation between the sedimentation characteristics of cyclic AMP-binding proteins and protein kinase and responsiveness to db cyclic AMP treatment was found with db cyclic AMP-responsive and -unresponsive 5123 hepatomas (data not shown).

Our studies have revealed a striking parallel between heat stability of cyclic AMP-binding and arrest of growth due to exogenous db cyclic AMP. Moreover, a close correlation was found between the heat stability of cyclic AMP-binding and affinity of the binding proteins for cyclic AMP: the binding proteins which are more stable to temperature possess a higher affinity for cyclic AMP than heat labile binding proteins. These qualitative differences of cyclic AMP-binding proteins between db cyclic AMP-responsive and -unresponsive tumours also reflect their capability for nuclear accumulation: only those binding proteins which are heat stable to temperature and exhibit a higher affinity for cyclic AMP accumulate into nuclei during db cyclic AMP treatment. These findings indicate that 'unresponsiveness' to db cyclic AMP treatment is closely associated with qualitative defects in cyclic AMP binding proteins. Thus the heat stability test of cytosol cyclic AMP-binding proteins could be used as a simple diagnostic method to assess the efficacy of cyclic AMP treatment of tumours.

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Prevention of senescence in the ascomycete *Podospira anserina* by the antibiotic tiamulin

THE syndrome of senescence or aging is common to all living material. In many fungi the peripheral hyphae become senescent after prolonged vegetative growth, that is, their growth rate decreases and they eventually die. In some fungi, senescence is caused by transmissible elements, but temporarily if their metabolism is drastically reduced^{1,2}, but

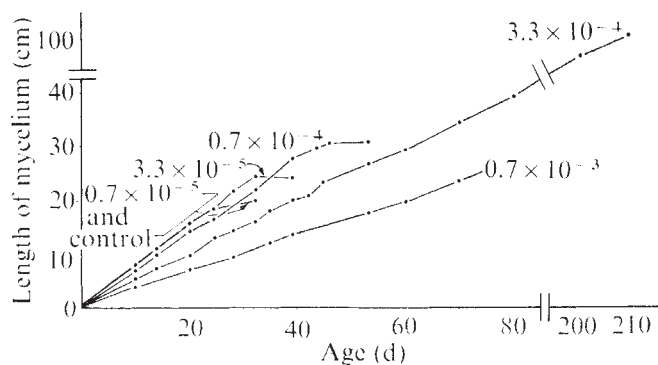


Fig. 1 Effect of the antibiotic tiamulin on the growth of *P. anserina*. Mycelia of the wild strain s_1 were grown at 25 °C in race tubes (length 50 cm) on cornmeal agar containing tiamulin as indicated. The growth curves were calculated from at least five parallel runs which were inoculated with mycelia 'freshly' regenerated by sexual propagation.

anastomosis. The onset of senescence is influenced by environmental as well as genetical factors¹⁻⁶. In *Podospira anserina*, where senescence has been very thoroughly analysed, it was shown that senescent mycelia may recover temporarily if their metabolism is drastically reduced^{1,3}, the mycelia soon become senescent when grown in normal conditions. The nature of the senescent agent in *Podospira* is obscure. All efforts to demonstrate viruses or virus-like particles in aging hyphae of *Podospira* have failed (R. Bozarth, personal communication). Our endeavours to determine the nature and action of the senescent agent were encouraged by previous findings that the onset of senescence

can be completely prevented by a synergistic action of specific genes⁶. In this paper we show that an antibiotic, tiamulin, can mimic the effect of these genes.

Informative experiments showed that when some tetracyclines were incorporated into the agar medium they induced a 'stopper phenotype'³, thus prolonging the life of the mycelium. In contrast, cholesterol reduced the life span by 30–50%. It is known that tetracyclines inhibit and cholesterol promotes the growth of mycoplasmas^{7,8}. It is thus possible that these organisms could be involved in the senescence syndrome in *Podospira*.

This idea is substantiated by the results of infection experiments. When senescent and juvenile mycelia were mixed in a Waring blender and incubated at 25 °C for 24 h, a 100% infection was obtained only in media with a high osmotic value (20% sucrose); at low osmotic values (1–5% sucrose) few or no juvenile hyphae were infected. This

Fig. 2 Correlation between the length of time the mycelium has grown on tiamulin and the onset of senescence. *a*, Time of linear growth of samples taken at various intervals (arrows) and transferred to medium devoid of tiamulin. *b*, Growth curve of the mycelium kept on tiamulin (3.3×10^{-4} M). For experimental conditions see legend to Fig. 1.

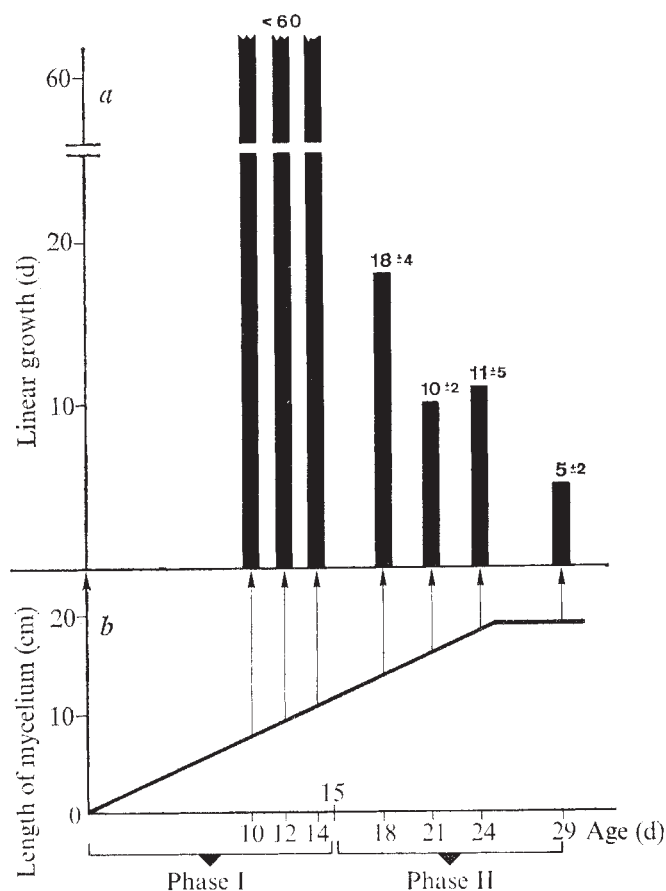
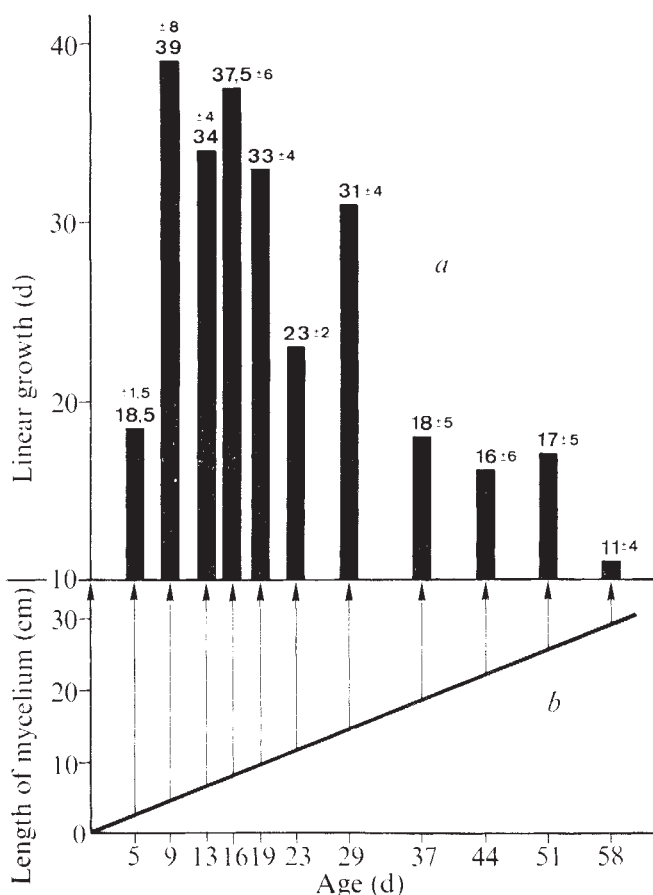


Fig. 3 Correlation between the age of mycelium and its sensitivity to tiamulin treatment. *a*, Time of linear growth of samples taken at various intervals (arrows) and transferred to medium containing 3.3×10^{-4} M tiamulin. *b*, Growth curve of mycelium grown on medium devoid of tiamulin. For experimental conditions see legend to Fig. 1.

osmotic sensitivity of the senescent agent is well in accordance with the properties of mycoplasma-like organisms (prokaryotic cells devoid of a cell wall).

In order to confirm this assumption we have looked at the effect of a new antibiotic, tiamulin, on the hyphal growth of *Podospira*. This antibiotic is supposed to be specific for mycoplasmas (personal communication Biochemie Kundl, Österreich). As seen from Fig. 1, tiamulin can prevent senescence. The most effective concentration is around 10^{-4} M. Lower concentrations only postpone senescence whereas higher concentrations prevent senescence but have a toxic effect and reduce the linear growth rate. In order to obtain more information on the action of tiamulin we have performed two series of experiments

(Figs 2 and 3). As can be seen from the data in Fig. 2, the time at which the onset of senescence occurs is influenced by the period for which the mycelium is first grown on tiamulin medium. There is considerable delay up till 30 d after which the situation is reversed and the onset of senescence soon occurs. Possibly the mycelium becomes dependent on tiamulin at this time. It is evident that tiamulin

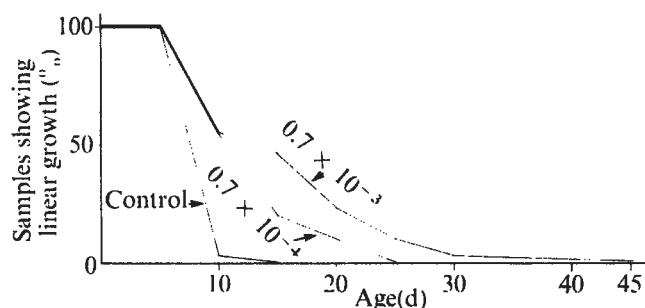


Fig. 4 Effect of various concentrations of tiamulin on senescent mycelium. Senescent mycelia grown in corn steep liquor in Fernbach flasks were homogenised in a Waring blender (60 s) in a solution of 20% sucrose containing tiamulin as indicated and incubated at 25 °C for 24 h. The life span of at least 30 samples at each concentration was followed after plating small inocula on corn meal agar. The curves are mean values showing the relative survival rates.

does not cure senescence but can prevent it when present in the medium.

Figure 3 shows that the life span of *Podospira* can be roughly divided into two phases with respect to the life-prolonging effect of tiamulin. If given in phase I (0–15 d) senescence can be prevented. If given about 10 d before the onset of senescence (phase II, 15 d and later) there is no longer an effect; the mycelium continues to grow on the antibiotic for the rest of its normal life span.

These observations confirm earlier findings¹ that even before these symptoms of senescence become obvious, the senescent agent is present in the mycelium. These data allow us to conclude that tiamulin can only prevent the activity of the senescent agent up to a critical time (perhaps a particular stage of development of the agent or its concentration in the cell). After this time the agent spreads within the mycelium and after an appropriate 'incubation time' causes the senescent phenotype.

To show that the ineffectiveness of tiamulin in phase II is not a result of its reduced uptake by the aerial hyphae, a 'therapeutic' experiment was performed in liquid culture. From the data summarised in Fig. 4 it can be seen, that tiamulin is still effective to a certain extent on senescent mycelium and that this effect increases with the concentration of the antibiotic. Two interpretations are possible. First, tiamulin prevents the infection of the few juvenile hyphae still present in the senescent mycelium and allows them to grow despite the presence of neighbouring senescent hyphae. Second, tiamulin may cure infected but not severely damaged hyphae and give them a new chance to grow.

Our experiments have shown that tiamulin is effective in preventing the occurrence of senescence in *Podospira* in specific conditions. As yet we do not know whether tiamulin acts prophylactically or therapeutically, but the possibility that it is acting by killing mycoplasmas must be investigated, particularly since mycoplasma-like organisms have recently been found in a fungus². The feasibility of using tiamulin and other derivatives of pleuromulin for preservation of strains and prolongation of fermentation time in the fermentation industry is under examination.

We wish to thank T. Itoh (Obihiro/Hokkaido, Japan) who contributed to the informative experiments with

tetracycline and cholesterol during his stay in our laboratory as a fellow of the Heinrich-Hertz-Stiftung (Düsseldorf). Tiamulin (2-diethyl-aminoethyl)-thioacetoxymutillin-hydrogenfumerat) was kindly donated by Biochemie G.m.b.H. Kundl, Österreich. We thank the technical staff of our laboratory for their assistance, especially Frau U. Rojek and Frau H. Hüsemann.

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Solubilised viral proteins produce fatal hepatitis in mice

PATHOGENICITY of viruses is commonly related to their ability to multiply within the host's cells. Although, in certain conditions, the inoculation of large doses of uninfected virus can produce a fatal disease¹, the toxicity of viral components as such for laboratory animals has never been demonstrated. We have shown that although frog virus 3 (FV 3) is unable to multiply in mice, it produces an acute degenerative hepatitis leading to the death of the animals in less than 24 h (refs 2, 3). We report here that solubilised viral proteins also produce this hepatotoxic effect.

FV 3 structural proteins were solubilised in two steps. First, a purified virus suspension was incubated with Nonidet P-40 (final concentration 0.5%) for 5 h at room temperature. The detergent was then removed through extensive dialysis in 50 mM Tris-HCl, pH 8.5. Second, LiCl (20%, w/w) was added to the preparation and the mixture was left for 6 h at 0 °C then dialysed again and centrifuged at 100,000g for 1 h. The supernatant fluid containing the soluble viral extract (SVE) was used in experiments⁴.

Intravenous (i.v.) inoculation of 0.2 mg of SVE proteins per mouse killed the animals within 24–48 h (Table 1): the

Table 1 Lethal effect of the solubilised viral extract in mice

SVE proteins (mg kg ⁻¹)	Treatment of SVE	Animals killed animals inoculated
10	None	6/6
5	None	5/6
2.5	None	5/6
1.25	None	1/6
0.62	None	0/6
20	80 °C, 30 min	0/6
20	Trypsin*	0/6
20	Tris-HCl	6/6
20	FV ₃ antibodies†	0/6
20	Normal rabbit serum	6/6

IC strain mice (18–20 g) were inoculated i.v. with 0.5 ml containing various amounts of viral proteins diluted in 50 mM Tris-HCl, pH 8.5, 15 mM NaCl.

*SVE (1.5 mg ml⁻¹) was incubated for 1 h at 37 °C with or without trypsin (0.5 mg ml⁻¹). Before the inoculation, 100,000 peptidase inhibitory units of iniprol (Choay) were added per ml of hydrolysed or unhydrolysed SVE.

†FV 3 antibodies were produced by inoculating rabbits with purified virus supplemented with complete Freund's adjuvant. SVE was mixed with anti FV 3 serum (final serum dilution 1/100) or normal rabbit serum for a 1-h incubation at 37 °C.

LD₅₀ was about 2 mg per kg body weight. This lethal action was related to SVE proteins. Both heating the SVE for 30 min at 80 °C and trypsin digestion destroyed its toxicity. The toxicity could also be neutralised by specific FV 3 antibodies (Table 1).

Histological lesions were found only in the liver. The whole lobule was affected with the exception of one or two layers of cells around the centrilobular vein. These lesions were studied by electron microscopy at intervals after the inoculation of one LD₅₀ of SVE. As early as 3 h afterwards, the nuclei of the hepatocytes were severely damaged; they showed an irregular shape, condensation of the chromatin, assembly of the interchromatin granules in dense clusters at the centre of the nucleus, and fibrillar inclusions (Fig. 1a).

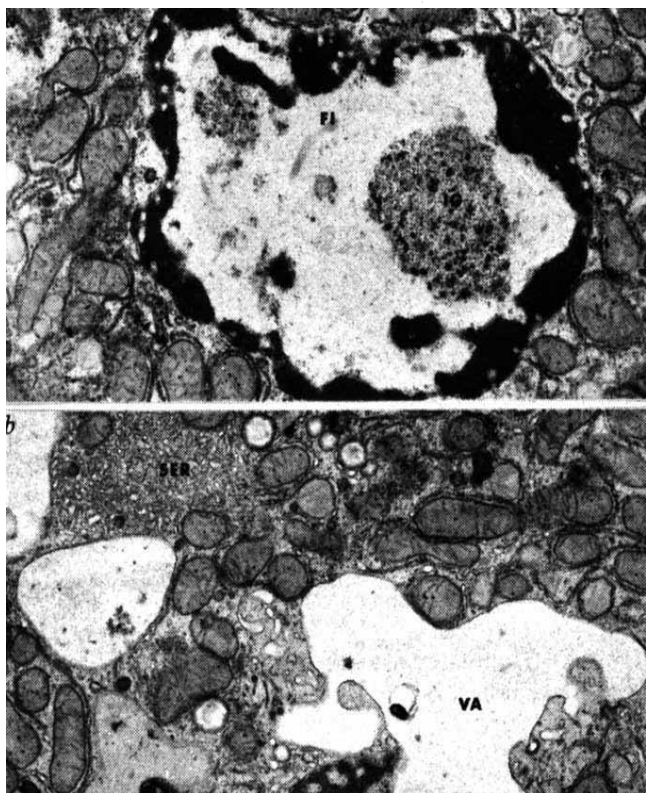


Fig. 1 Ultrastructural changes produced in hepatocytes of mice inoculated i.v. with SVE 10 mg per kg body weight. *a*, Hepatocyte nucleus observed 3 h after the inoculation. The condensation of the chromatin (CH) and the emptiness of the nucleoplasm appear clearly. The interchromatin granules (IG) are assembled into a dense cluster at the centre of the nucleus and some fibrillar inclusions (FI) are noticeable. $\times 7,200$. *b*, Part of the cytoplasm of a hepatocyte 9 h after inoculation. The cytoplasm shows numerous vacuoles (VA) as well as large areas of smooth endoplasmic reticulum (SER) ($\times 9,1120$).

Cytoplasmic changes were only noticeable 9 h after the inoculation. They were characterised by alterations of both rough and smooth endoplasmic reticulum, accumulation of lipids and a final necrosis (Fig. 1b).

As with inoculation of virus particles, the macromolecular metabolism of the liver was markedly affected by SVE injection. Table 2 shows that both RNA and protein synthesis were depressed 3 h after inoculation, RNA synthesis being more severely affected.

Thus the inoculation of solubilised structural proteins from FV 3 produces the same morphological and biochemical alterations of the liver as the intact virions. Our results clearly demonstrate that viral proteins may be 'toxic' by themselves. Although it is unlikely that an organism would be naturally infected with an amount of virus sufficient to produce extensive lesions by this mechanism, most pathogenic viruses undergo several cycles of multiplication enabling toxic proteins to accumulate.

Table 2 Effect of SVE on RNA and protein syntheses in mouse livers

Time after inoculation (h)	c.p.m. incorporated	
	³ H-uracil	¹⁴ C-amino acid
0	147,680 (100)	269,520 (100)
3	81,200 (55)	180,560 (67)
6	59,040 (40)	161,680 (60)
9	51,680 (35)	134,760 (50)

Mice were inoculated i.v. with SVE at 10 mg per kg body weight. The mice received 50 μ Ci of ³H-uracil (specific activity 22 Ci mmol⁻¹) and 10 μ Ci of ¹⁴C-amino acids (specific activity 0.660 mCi mmol⁻¹) intraperitoneally 30 min before killing. The livers were removed rapidly and homogenised in 5 ml of 250 mM sucrose. RNA and protein synthesis were measured as incorporation of ³H-uracil and ¹⁴C amino acid respectively into the acid insoluble material of the liver homogenates. The results are expressed in c.p.m. per g of wet liver, each value representing the mean obtained with four animals. Figures in parentheses indicate the percentage of incorporation into the livers of the inoculated mice compared to the incorporation into the livers of the untreated animals.

The model described here should be of use in the study of the mechanisms responsible for the shutoff phenomenon and cell necrosis during lethal virus infection. Since non-replicative viral material can be highly toxic, the part played by the competition for essential metabolites as a factor in cytopathogenicity can in this case be ruled out.

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Granulomatous hypersensitivity induced by sensory peripheral nerve

THE nature of granulomas remains uncertain. In sarcoidosis and Crohn's disease of the ileum, the aetiology is unknown. Even where a causative organism has been identified, as in tuberculosis and non-lepromatous leprosy, the role the mycobacteria play in the development of the granuloma is obscure¹. There is no animal model available for the study of granulomatous hypersensitivity. But in susceptible human subjects it is possible to produce granulomas with tubercle formation by skin testing with zirconium² or beryllium³. Similarly, in sarcoidosis, granulomas with tubercles occur in patients skin-tested with Kveim antigen (sarcoid spleen or lymph node suspension). These reactions are specific for each antigen and independent of dose. Thus, chronic states of altered tissue reactivity or granulomatous hypersensitivity can be found in humans⁴. We have shown that rabbits develop skin lesions after injection with homogenates of peripheral sensory nerve⁵. Now we report that these lesions sometimes possess organised granulomas in tubercles, and that similar granulomas can be produced by skin-testing rabbits sensitised with sensory nerve, using cutaneous nerve as the challenging antigen. These results suggest that granulomas may sometimes be an autoimmune response to tissue antigens: in the case of non-lepromatous

leprosy, to an antigen in peripheral nerve containing sensory fibres⁵. A specific diagnostic test for leprosy is therefore a possibility.

It is important to distinguish granulomas from foreign body and delayed-type hypersensitivity reactions, and we have used the following criteria. First, the granuloma should be organised in tubercles, with epithelioid cells located centrally, surrounded by lymphocytes. Second, the putative epithelioid cells should possess the histological features of the epithelioid cells found, for example, in zirconium-induced granulomas, that is they should be large pale staining clear cells, having prominent nucleoli, with the chromatin of large ovoid nuclei lying peripherally¹. Third, it should be possible to produce the granuloma by challenge with antigen alone at sites remote from the original site of injection of antigen plus Freund's adjuvant, that is a state of granulomatous hypersensitivity should exist⁴. Fourth, electron microscopy of the cells should show evidence of secretory activity, with abundant rough endoplasmic reticulum in the cytoplasm, to distinguish between the secretory epithelioid cells and macrophages⁶.

Skin patches developed systemically in 14 out of 20 Dutch bantam rabbits injected with sural nerve plus Freund's adjuvant as described previously⁵. It is important to stress that none of these patches developed at the site of the injections. In four of the responding rabbits histological features of granuloma were found in the patches which had formed on the dorsum of the foot, the footpad of which had been the site of the original injection. Tubercles and developing tubercles with centrally placed epithelioid cells surrounded by lymphocytes could be seen. Four rabbits given Freund's adjuvant alone and observed for 7 months did not develop these skin patches.

A second group of rabbits was used for the skin testing experiments. Initial sensitisation was by injection into one hind footpad of 0.2–0.4 ml of a homogenate of human peripheral nerve plus Freund's complete adjuvant, prepared as described previously⁷. Three different types of peripheral nerve were tried, sural (cutaneous), dorsal roots (sensory fibres) and ventral roots (mainly motor fibres). Subsequent skin testing in all cases was with 0.2 ml 10% human sural nerve homogenised in saline and injected intradermally into the back. The sensitisation and challenge regimen is summarised in Table 1.

Papules (5–10 mm in diameter) appeared in seven out of 18 rabbits skin-tested and these papules persisted until biopsy at 5 weeks. In the negative cases swellings were transient, not lasting beyond 2 weeks. In six out of the seven animals which developed papules, histological features of granulomas were found, that is tubercles and developing tubercles with centrally placed epithelioid cells surrounded by lymphocytes could be seen (Fig. 1). The epithelioid cells were large and pale staining with the chromatin lying peripherally in the large ovoid nuclei. Prominent nucleoli were frequently present. Langhan's-type

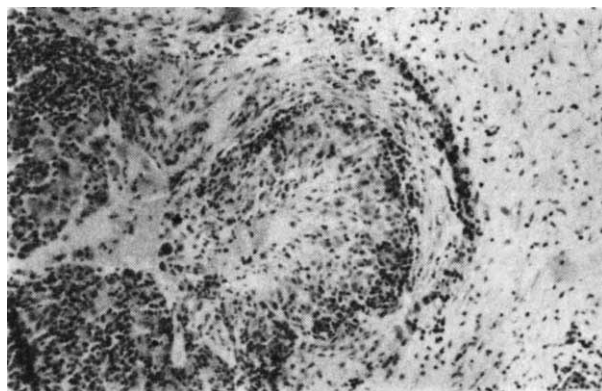


Fig. 1 Section of papule 5 weeks after intradermal injection of 0.2 ml 10% human sural nerve into back of rabbit, showing tubercle formation with epithelioid cells surrounded by lymphocytes. Stained with haematoxylin and eosin, $\times 92$. The rabbit had been injected with 0.2 ml sural nerve with Freund's adjuvant in the hind footpad 18 months before skin test.

giant cells with peripherally situated nuclei were present, the nuclei being similar in form to those of epithelioid cells⁸ (Fig. 2).

In two rabbits part of the biopsy specimen was fixed for electron microscopy. In both specimens typical epithelioid cells were found, that is the cytoplasm contained abundant

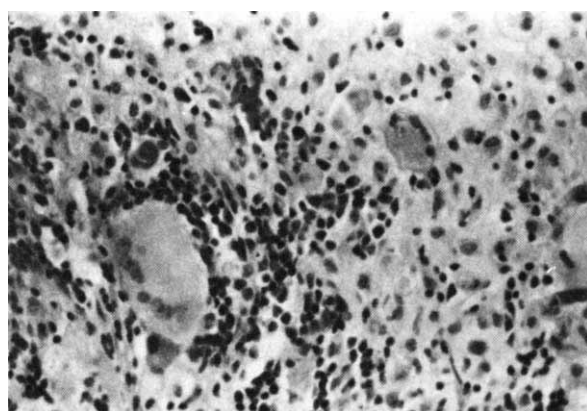


Fig. 2 Part of a developing tubercle with Langhan's-type giant cells and typical epithelioid cells, $\times 250$. The rabbit had been injected with 0.2 ml dorsal roots with Freund's adjuvant 14 and 13 months before the skin test with sural nerve.

dilated rough endoplasmic reticulum with a moderately dense secretory product; an active Golgi apparatus was also present (Fig. 3). These observations confirm the secretory nature of the cells.

Rabbits which failed to develop papules showed histologically only diffuse collections of cells with no organisation into tubercles or distinguishing features of epithelioid

Table 1 Granuloma induction with various types of human peripheral nerve as sensitising antigen

No. of rabbits	Sensitising antigen	Sensitisation schedule before challenge with 10% sural antigen	Animals showing granuloma
7	Sural	A single injection (0.2 ml)	4
5	Dorsal roots	13 months to 2½ yr	
		14 months 0.2 ml*	2 (1*)
		13 months 0.2 ml	
		11 months 0.2 ml	
4	Ventral roots	3 months 0.4 ml	
		11 months 0.2 ml	0
		10 months 0.4 ml	
2	None	3 months 0.4 ml	0
		—	

*One positive had only two injections at 14 and 13 months before challenge.



Fig. 3 *a*, Electron micrograph of part of a tubercule showing fully developed secretory epithelioid cells and undifferentiated mononuclear cells. The tubercule was taken from a skin papule 5 weeks after skin-testing with 0.2 ml 10% sural nerve in saline intradermally into the back of the rabbit previously injected with 0.2 ml sural nerve in Freund's adjuvant in footpad 2.5 yr before. Stain was lead uranyl acetate, $\times 3,440$. *b*, Electron micrograph of part of a cell possessing the characteristic organelles of a secretory epithelioid cell. From the same tubercule as (*a*). GER, granular endoplasmic reticulum containing secretory product. SGER, stacks of granular endoplasmic reticulum. G, Golgi apparatus. N, nucleus. Stained with lead uranyl acetate, $\times 24,395$.

cells. Injection of 0.2 ml 10% rat spleen or rat brain did not produce any papules in five out of six rabbits showing the positive granulomatous response to challenge with sural nerve; no histological features of granuloma were seen. A total of 32 skin tests, using rat spleen and rat brain as challenging antigen, produced no papules in these rabbits.

Central necrosis in tubercles was common in both spontaneously occurring and skin-test-induced granulomas which occasionally led to small abscess formation. Necrotic foci are common in beryllium granuloma^{8,9} and in strongly reacting patients the Kveim test sometimes shows necrosis^{10,11}.

Thus we have induced granulomas and a state of granulomatous hypersensitivity in 10 rabbits and it seems that the antigen is located in peripheral nerves containing sensory fibres.

A viable agent has been postulated as the cause of sarcoidosis and Crohn's disease, and samples of affected tissue have been inoculated into animals in an attempt to reproduce the disease^{11,15}. Claims for successful attempts to reproduce Crohn's disease have not been confirmed¹⁵. Recently a virus has been isolated from ileal tissue affected

by Crohn's disease, but this has not yet been shown to cause Crohn's granuloma. A viral agent, by modifying the cell surface antigen composition, could produce an autoimmune response.

In none of the experiments have the animals developed a state of granulomatous hypersensitivity, that is, skin testing at remote sites has not induced granuloma formation. Our results suggest that an autoimmune approach using tissue antigens from the ileum may be effective. The experimental model described here may be useful in testing drugs for use in sarcoidosis and Crohn's disease.

By inducing granulomas in rabbits we have reproduced the histological features of non-lepromatous leprosy; this supports previous work² suggesting that the skin lesions in the human disease are autoimmune responses to sensory peripheral nerve rather than being directly due to *Mycobacterium leprae*. It may also be possible to devise a skin test specific for leprosy, using sensory nerve as antigen. This test would be similar to the Kveim test for sarcoidosis, but would differ fundamentally from skin tests using the antigen *M. leprae* (lepromin) which are of no diagnostic value, giving foreign body reactions in both healthy people and those with non-lepromatous leprosy.

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Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials

PERIPHERAL AXONS retain into adult life the ability to grow, and do so either if they themselves are cut or if neighbouring tissues are denervated (collateral sprouting¹). Collateral sprouting also occurs in botulinum toxin poisoning², motor end plate disease of mice³ and myasthenia gravis of man⁴. Muscle inactivity is common to these otherwise different conditions. We have therefore investigated whether inactivity in a muscle, produced by a local nerve block, will give rise to collateral sprouting. By paralyzing motor nerves for a few days with a local application of tetrodotoxin (TTX) we have found that motor nerve terminals in the affected muscles do grow collateral sprouts. We conclude that muscle inactivity results in a sprouting stimulus to nerves.

TTX was used because it is a specific blocker of Na⁺ conductance in nerve⁵ and does not interfere with axoplasmic transport^{6,7}. This is important because Diamond

et al.^{8,9} believe that interference with axoplasmic flow can lead to sprouting. Adult mice, male and female (20–30 g), were anaesthetised with ether, the right sciatic nerve was exposed and a hollow cylindrical cuff of Silastoseal B (Midland Silicones) was placed carefully around the nerve. Cuffs were 1.5–3 mm long, 2 mm in diameter with a 1-mm diameter hollow core. They were made as described by Lavoie *et al.*⁶, except that the quantities of TTX and NaCl were different. In the first experiments the cuffs contained approximately 1 µg of TTX and 3 mg of NaCl. These paralysed for 24–48 h and two or three had to be used to give sufficient paralysis. In later experiments the cuffs contained about 5 µg of TTX and 0.5 mg of NaCl, and before insertion they were sprayed inside and out with poly tetrafluoroethylene (PTFE, DMC Industrial Aerosols, No. 889RB). This delayed release of TTX and allowed larger doses to be given, thus prolonging paralysis, but often the paralysis was not as profound as with the other type of cuff. Control animals were mice with cuffs containing no TTX and mice with TTX-containing cuffs which paralysed for only 1 or 2 d; some of these were coated with PTFE, others were not. The ineffective cuffs were left *in situ* for a further 3 or 4 d to bring the period when the cuffs were present up to that for animals where paralysis lasted for 3.5–7 d. Paralysis was checked twice a day by observing the movement and stance of the mice and by picking them up by the tail to see if their toes spread normally. Paralysis was not always complete but provided it was marked it was counted as successful. Different cuffs of a given batch did not necessarily paralyse to the same extent. We attributed this to the difficulty of mixing the small quantities of TTX, NaCl and silicone rubber into a uniform paste, so that different amounts of TTX were probably present.

Animals were used for the acute experiment within 24 h of the disappearance or marked diminution of paralysis. The peroneus tertius and solar muscles together with their nerves were dissected out of the right and left leg and studied *in vitro* at room temperature (normally 20 °C), perfused continuously with well oxygenated fluid of the following composition: 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 5 mM CaCl₂, 1.24 mM HEPES, pH 7.4, 16.5 M glucose. Maximum isometric twitch and tetanic (50 per s) tension was recorded on stimulation of the nerve below the block. These tensions were compared with that evoked by direct stimulation of the muscle fibres (stimulus applied by two

bright silver wires on either side of the muscle). The direct stimulus intensity was turned up gradually and usually maximum tension was produced by 10-V, 100-µs pulses. A 20-V, 300-µs pulse was then used to check that all the muscle fibres were being stimulated. If the direct and nerve-evoked tensions were not identical, the muscle was discarded as some axons had presumably been killed. This occurred on four out of 13 occasions in peroneus tertius, but not at all in 13 soleus muscles. When the two tensions were identical, a further check for absence of denervation was to grade the stimulus to the muscle nerve and, by sequentially bringing in motor units of different threshold, to count them. The number counted in this way in the experimental soleus and peroneus tertius was then compared with the figure for normal muscles from the opposite leg. When the direct tension equalled the nerve evoked tension, the unit numbers counted in this way were the same on the normal and experimental sides. The mean unit number for peroneus tertius was 11 and for soleus 21. The muscles were then curarised and end plate potentials were recorded with conventional 10 MΩ glass micropipettes. We sometimes had to use up to five times the normal dose (5 × 0.5 µg ml⁻¹) of *d*-tubocurarine to block transmission in the experimental muscles. The recording of end plate potentials enabled us to check first that all fibres were innervated and second, by grading the stimulus to the nerve, whether as a result of sprouting any fibres had become polyneuronally innervated. Twenty to fifty fibres were examined in each muscle. Finally the preparations were stained for 5–6 h in a 4:1 solution of zinc iodide and osmium tetroxide¹⁰, washed in water, teased with forceps and scissors into fine bundles, dehydrated, cleared in xylol and mounted in Permount.

Provided paralysis had lasted for 3.5 d or longer the histology showed fine ultraterminal sprouts extending from the motor nerve terminals. In some cases the fine branches were very short (Fig. 1*b*) and in others extensive outgrowths could be seen (Fig. 1*c*). The soleus endplates sprouted more vigorously than those in peroneus tertius which usually had only a single large sprout. This is in agreement with results from sprouting induced by botulinum toxin¹¹. Five control animals with non-paralysing cuffs had normal endplates (Fig. 1*a*). To obtain an objective figure for the degree of sprouting, we measured for 50 endplates in each muscle, first the length, in the long axis of the muscle fibre, of the nerve

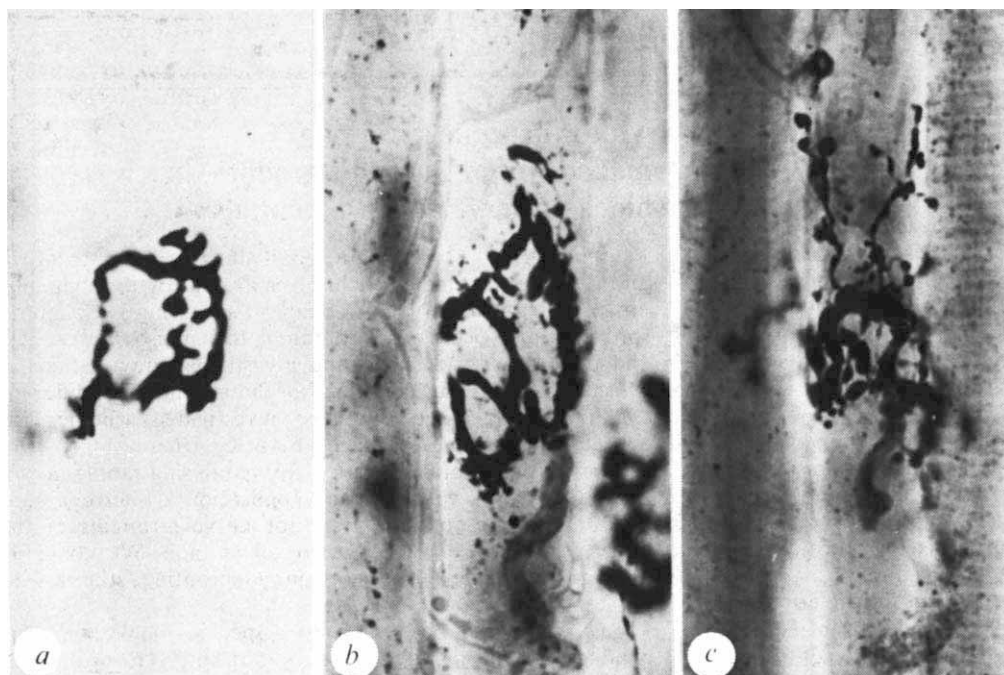


Fig. 1 Photomicrographs of mouse soleus endplates stained with zinc iodide and osmium tetroxide⁷ from: *a*, a control animal in which a nonparalysing cuff was present for 5 d; *b*, paralysis for 3.5 d; *c*, paralysis for 5 d. The calibration bar on the right is 50 µm.

terminal which is densely stained by zinc iodide and osmium tetroxide, and second in those endplates with sprouts the length of endplate plus the furthest sprout extensions in both directions along the muscle fibre. Sprout length was taken as the difference between these two measurements. The percentage of endplates with sprouts in each experiment is shown in Fig. 2a, for paralysed, cuffed but not paralysed, and for normal muscles. Figure 2b shows, for each experimental muscle, the extent of sprouting (μm per endplate) obtained by dividing the total length of sprouts measured for that muscle by the number of endplates measured. For the control muscles the figure is near zero. As described by Barker and Ip¹², a small amount of sprouting is seen in normal muscles.

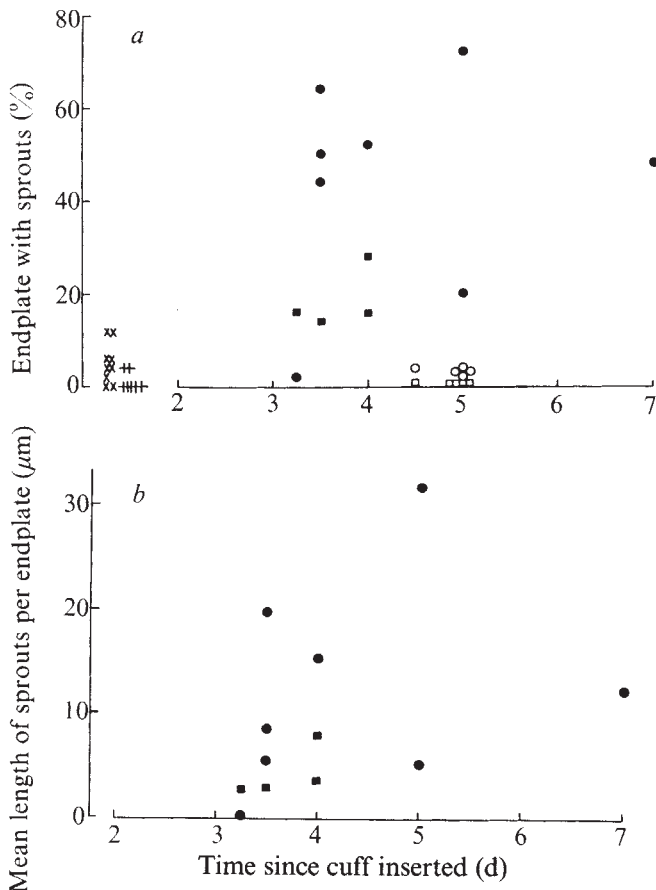


Fig. 2 a, Percentage of endplates with ultraterminal sprouts; b, mean length of sprouts per endplate (see text); ●, paralysed soleus; ■, paralysed peroneus tertius; ○, control soleus; □, control peroneus tertius; ×, normal soleus; +, normal peroneus tertius.

Even in muscles with much sprouting some endplates appeared quite normal. Our test for paralysis was not very sophisticated and it seems likely that not all the motor units were blocked for all the time, and it may also be that within one muscle the latent period before sprouting starts differs between motor units, as it does between units in different muscles¹¹. Less than 2% of the fibres were found to be polyneuronally innervated by our physiological test. As a similarly very small proportion of normal mouse soleus fibres is polyneuronally innervated, it seems that the blocking times we have achieved are not long enough for sprouts to form functional endings on other fibres in significant numbers. If we can achieve longer blocks in future it will be interesting to see if they do and what their fate will be when paralysis wears off. In both normal and experimental muscles we failed to record endplate potentials in about 3% of fibres impaled.

These results show, in agreement with others^{6,7} that TTX can be used to paralyse without killing nerves, and as control animals with cuffs showed no more than normal sprouting¹² any interference of the cuff *per se* with axoplasmic transport was not enough to cause sprouting. We therefore conclude that a few days inactivity of muscle gives rise to a sprouting stimulus. The work of Lomo^{13,14} *et al.* has shown that inactivity induced by a local anaesthetic nerve block causes changes in the muscle membrane similar to those of denervation and this result has been confirmed by using TTX block^{6,7}. The denervation changes can be prevented by direct stimulation of the muscle^{13,14}. We have found (unpublished observations with G. M. Goodwin) that induction of sprouting by botulinum toxin can be prevented by direct stimulation of the muscle. Inflammation also causes muscle membrane hypersensitivity and can cause nerve sprouting¹⁵. It seems that sprouting is induced by a stimulus from the muscle which may be present normally to a small degree, but is greatly increased in various conditions; for example during prolonged inactivity or inflammation. Whether the stimulus is a diffusible chemical, or a change in the surface of tissues surrounding the nerve terminals is unknown.

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Punishment learning and self-mutilation in Lesch–Nyhan disease

THE self-injurious behaviours (SIB) of Lesch–Nyhan children were studied during three learning paradigms: punishment, positive reinforcement and time-out from reinforcement following an attempt at self-injury. Punishment of self-injury by contingent electric finger shock failed to suppress these behaviours but positive reinforcement of non-self injury and time-out procedures were effective. The results stress the importance of environmental factors in the development and control of SIB and suggest the presence of an unusual learning defect which may be related to the specific enzyme deficiency in Lesch–Nyhan disease.

Lesch–Nyhan disease is an autosomal recessive disease in which the primary defect is in the synthesis of a catalytically ineffective enzyme, hypoxanthine phosphoribosyltransferase^{1–3}. A most striking aspect of this disease is the bizarre pattern of self-mutilation that appears at about two years of age. Finger biting is characteristic. This may cause severe injury, even amputation. Elbow splints and hand bandages may have to be worn continuously to prevent damage. If the restraints are removed, the patient screams in apparent panic, ‘compulsively’ raises his hand to his mouth and will bite his fingers. Once securely restrained, the patient again becomes placid. Lip biting may also develop, requiring extraction of teeth. It should be noted

that the children do not appear to be deficient in pain sensitivity, and are less retarded than originally thought⁴. Many biochemical approaches to treatment have been tried without success⁵.

Five males, ages 3, 5, 11, 12 and 13 yr, with the characteristic clinical and biochemical findings of Lesch-Nyhan disease served as subjects. Before treatment all wore arm restraints and exhibited scars on fingers and lips. A standardised assessment procedure was devised to establish a baseline rate of SIB while avoiding extensive injury to the child. At the beginning of the baseline period the child is seated in a wheelchair and his arms are released from the restraints (a splint that prevents the elbows from bending). An assistant seated in front of the child intercepts the hand as it nears the mouth and places it back in the child's lap without speaking or otherwise interacting with the child (response prevention). Observers out of the child's line of vision record the behaviour. For a summary of the treatment procedure and results see Fig. 1.

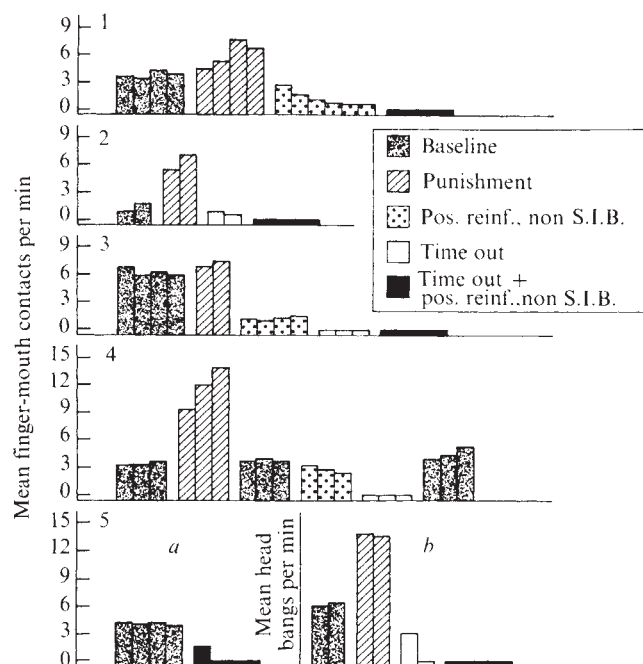


Fig. 1 Response of five Lesch-Nyhan children to treatment for SIB. Punishment does not decrease the frequency of undesirable incidents. Positive reinforcement of non-self injurious behaviour reduces the frequency; the best results are achieved with time-out and time-out plus positive reinforcement of non-SIB. Each bar represents the mean average SIB response rate per min during a session about 30 min long. Usually three sessions were conducted per day. The children's data are presented in the order of admission to the hospital.

'Aversive stimulation' has been the most widely used approach to the control of self-injury⁸⁻¹². In the punishment paradigm, aversive stimulation is delivered immediately following the undesirable behaviour. Consent of the Human Research Committee was obtained and parents experienced the electric shock before agreeing to the treatment plan.

Subjects 1-4 were given electric finger shock contingent on each hand-to-mouth contact. The fifth child was given shock following each head bang. In all cases the shock intensity was 5 mA (a level generally described as quite painful). The results of the punishment procedure can be seen in Fig. 1. In all cases the procedure failed to suppress the frequency of SIB and, in fact, seemed to promote such behaviour.

Through naturalistic observation of parents and child it was determined that the frequency of SIB varies considerably and predictably with variations in the environment

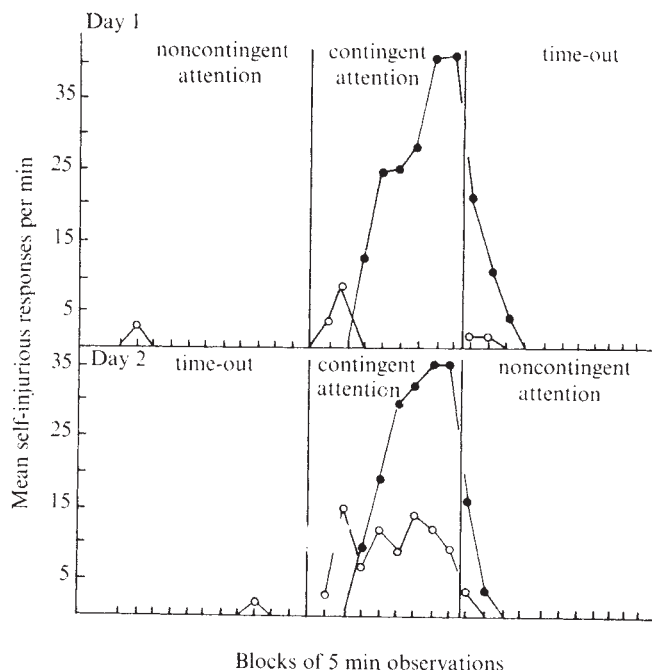


Fig. 2 SIB rate in response to non-contingent attention; contingent attention; and time-out. Each data point represents the mean number of finger biting (○) or lip biting (●) responses per min, for blocks of 5 min observations. For each of the two days the session was continuous (3 h) with no pause between manipulations.

suggesting a learned behaviour that is under discriminatory control.

The facilitory influence of positive reinforcement of SIB can also be clearly demonstrated under controlled conditions. The therapist was instructed to give contingent attention by quickly interceding to prevent self-injury while making reassuring statements, and stroking the child. The frequency of SIB, in one child so treated, increased dramatically when compared to non-contingent attention or time-out (Fig. 2).

Positive reinforcement of non-SIB and time-out, either alone or in combination consistently produced rapid reductions and eliminations of SIB. When the child was not agitated or attempting to bite, the therapist would reinforce these behaviours by smiling, talking, playing or in other ways interacting with the child. Time-out consisted

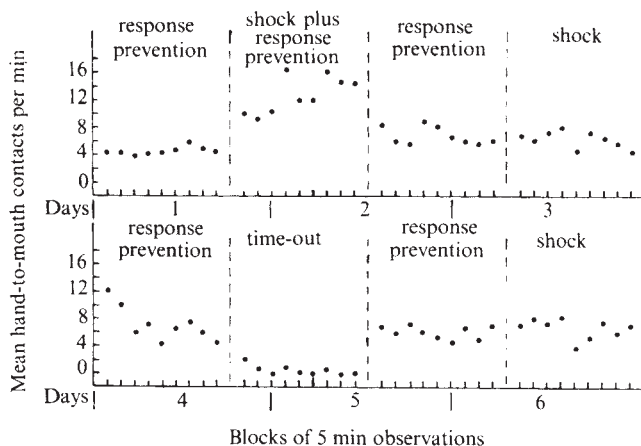


Fig. 3 Response of SIB to response interruption or baseline procedure; shock plus response interruption; shock alone; and time-out. Shock plus response interruption increases the SIB above baseline level. Shock alone produces no variation from baseline. Time-out reduces SIB to near zero. Each data point represents the mean number of hand-to-mouth contacts per min per 5 min observation period.

of the withdrawal of all attention to the child following each SIB attempt. The therapist simply turned away from the child for about 5 s. The response prevention procedure used during baseline of intercepting hand-to-mouth contacts was discontinued.

Because of the danger of self-injury, finger shock and the response prevention procedure always occurred together. This pairing of physical attention with finger shock could account for the increase in response rate during punishment. Before therapeutic training subject 5 was used to evaluate these two variables (Fig. 3). When shock is given without response interruption the SIB rate does not vary from baseline rates.

We have not shown in Fig. 1 the results of a programme for the maintenance of these effects. For all children, the rate of SIB remained at zero for approximately 3 months. At an 18-month follow-up the average SIB rate is 20% of initial baseline. Presently the behaviour is under a high degree of stimulus control with certain adults or specific settings producing complete absence of self-injury, while others produce SIB comparable to pretreatment rates.

An unexpected observation in these studies has been the inability to reduce or eliminate a particular behaviour by the administration of an aversive stimulus via the positive punishment paradigm⁶. This technique is so regularly effective under experimental and natural conditions, and consistently effective in the elimination of SIB in other diagnoses, that its ineffectiveness in Lesch-Nyhan disease was particularly striking.

One explanation is that Lesch-Nyhan children are incapable of learning from aversive stimulation. In contrast, they learn quickly from positive reinforcement and timeout, suggesting a specific learning disability related to an identifiable gene. Such an explanation will depend on whether or not the inability to learn from aversive experience is specific to SIB or a general effect that extends to other aspects of learned behaviour.

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Peri-ovulatory synchronisation of behaviour in male and female rhesus monkeys

Rhesus monkeys, like many other primates, do not restrict their sexual activity to the ovulatory period, and copulation may occur at any time during the menstrual cycle^{1,2}. Thus, in the laboratory, the male's ejaculatory frequency is generally highest near mid-cycle (although different pairs show different patterns of mounting and ejaculation³) while, in the wild, sexual activity seems to be more or less restricted to the middle part of the cycle^{4,5}. It is difficult to judge the stage of the cycle in troops of feral rhesus

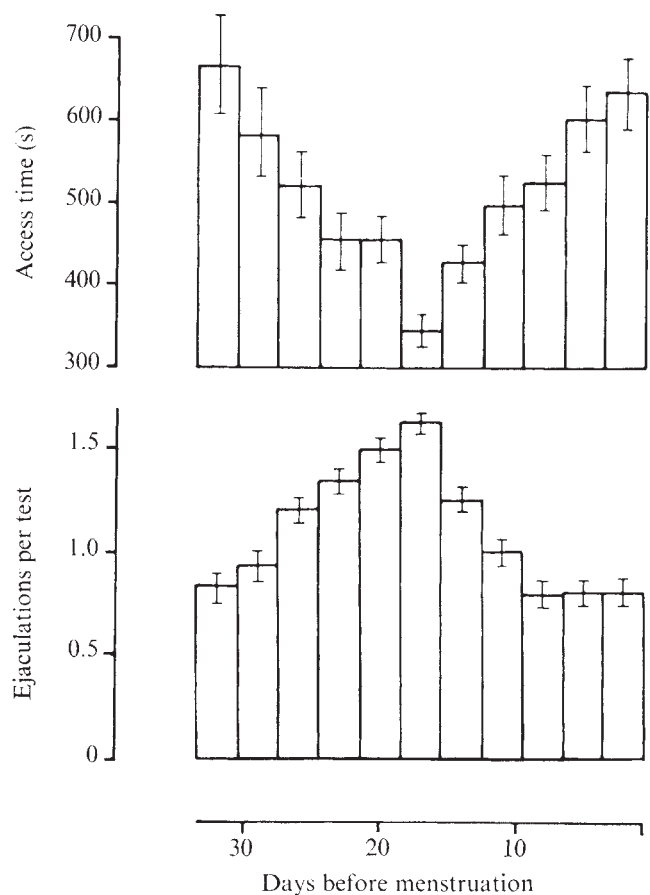
monkeys, but during the period of maximum sexual activity females actively approach males and form temporary consort bonds which involve mutual grooming and frequent copulations⁶. To obtain an independent and objective measure of female sexual motivation, we have used an operant conditioning technique in which the female was required to press a lever for access to her male partner. This has made it possible to relate changes in female motivation precisely to changes in the circulating levels of ovarian hormones. We have also demonstrated a synchronisation and maximisation of female sexual motivation with male ejaculatory performance during the peri-ovulatory (fertile) period of the menstrual cycle.

When pairs of rhesus monkeys are tested together in laboratory conditions, the close proximity and dominance of the male, nearly twice the size of the female in this species, strongly influences the female's behaviour, and it is difficult to obtain an independent measure of the female's sexual motivation uncontaminated by the threat of male aggression. Our operant conditioning technique was developed to avoid this problem by giving the females themselves control over their access to males⁷. The testing cages were divided by a movable partition, and pressing a lever 250 times on one side of the partition activated a servo-motor that raised it, facilitating access to the other side of the cage. Females were trained to perform this task, first for food reward and then, when they were familiar with the situation, for a male partner. The period from the introduction of the female into the cage to the moment the door opened (access time) was timed automatically. After the female obtained access to the male, a behaviour test of 1 h was conducted during which observers scored various aspects of the pair's behaviour from behind a one-way vision mirror⁸. If the female failed to obtain access in the 30 min allowed, animals were removed from the operant cage and, later the same day, were brought together in a different cage for a 1-h behaviour test; this preserved the continuity of the behavioural data.

Sixteen rhesus monkeys (nine females and seven males) were obtained directly from India. The Fallopian tubes of females were ligatured to prevent pregnancy. Results are based on 1,440 tests of 17 pairs conducted during 63 menstrual cycles (30.1 ± 4.8 d, mean $\pm$ s.d.). Data were combined by aligning the cycles on the first day of the next menstruation and calculating 3-d means. The frequency of ejaculation in these pairs followed a pattern similar to that found in earlier studies⁹, and showed a maximum (1.63 ± 0.05 ejaculations per test, mean $\pm$ s.e.) at the end of the first half of the cycle (reverse days 18-16), and a minimum (0.80 ± 0.07 ejaculations per test) in the middle of the second half of the cycle (reverse days 9-7) (Fig. 1). The shortest mean access time (342.3 ± 23.3 s) also occurred during reverse cycle days 18-16, and the longest mean access times occurred near the beginning and end of the cycle. The highest ejaculation frequency and the fastest access time therefore occurred at, or just before, the expected time of ovulation⁹. These data confirmed the preliminary results obtained with this operant conditioning technique⁷. Although the nature of the stimulus that motivated females to lever-press for access to males remains unknown, and may well vary from pair to pair, access times for males were not correlated with access times for food rewards on the same day or with access times when a female instead of a male was the reinforcement. Thus, we can assume that the access time data reflected the female's motivation for the male and were not merely dependent on cyclic changes in the female's motor activity.

To obtain more precise information on the temporal relationship between behavioural parameters and hormonal status, blood samples were collected from five females on alternate days (daily near mid-cycle) during 33 menstrual cycles. These samples were analysed for progesterone,

oestradiol, testosterone and dihydrotestosterone simultaneously using a radioimmunoassay that involved chromatography on Sephadex LH20. All these cycles appeared normal, with the characteristic pre-ovulatory oestradiol peak occurring 19–17 d before the next menstruation, and with rising progesterone titres signalling the development of corpora lutea. Hormonal and behavioural data from the periovulatory periods of these 33 cycles were then combined by aligning them on the days of the oestradiol peaks (Fig. 2). The highest mean ejaculation frequency and the fastest mean access time both occurred together 1 d after the oestradiol peak, and on that day all males always succeeded in ejaculating and all females always pressed for access to their partners. The statistical analysis of the

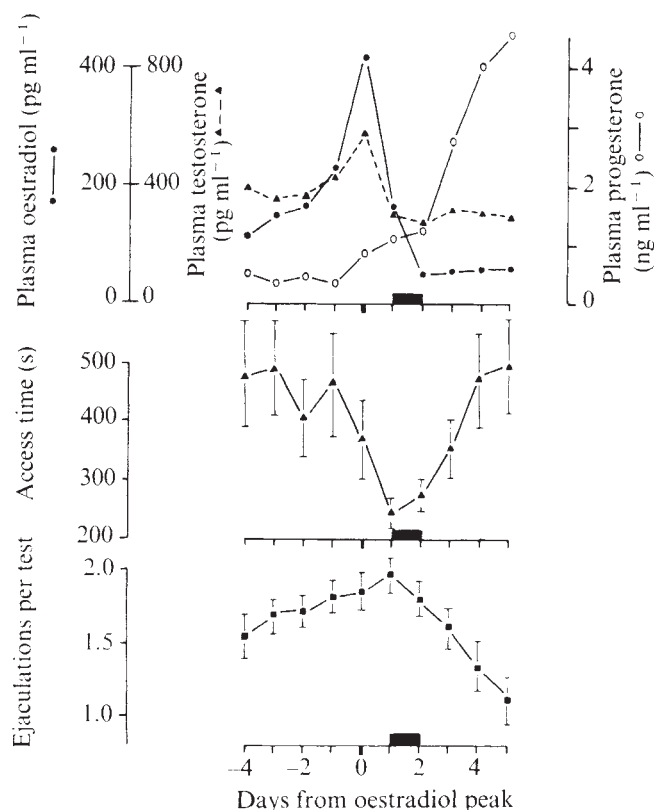


$N = 81 \ 85 \ 137 \ 131 \ 157 \ 136 \ 155 \ 139 \ 145 \ 142 \ 132$

Fig. 1 Changes in the speed with which female rhesus monkeys lever-pressed to gain access to males on different days of the menstrual cycle, and the relationship to the ejaculatory performance of their male partners. The 63 menstrual cycles were aligned on the first day of the next menstruation and data were calculated as 3-d means. The shortest mean access time and the highest ejaculation frequency both occurred at, or just before, the expected time of ovulation. Vertical bars show standard errors of means. N , number of tests (nine females, seven males, 17 pairs, 1,440 tests).

behavioural data (for cyclicity and synchrony) is necessarily complex and involves the treatment of 18 separate indices for each of the 17 pairs; results will be fully reported elsewhere.

Studies in which gonadal steroids were administered to ovariectomised rhesus monkeys showed that changes in the sexual behaviour of the pair can be induced by ovarian hormones³. Thus, the mid-cycle peak in plasma oestradiol, besides triggering the gonadotrophin surge responsible for



$N = 18 \ 20 \ 30 \ 19 \ 32 \ 25 \ 22 \ 28 \ 25 \ 25$
 $n = 24 \ 28 \ 28 \ 26 \ 26 \ 19 \ 20 \ 25 \ 29 \ 27$

Fig. 2 Changes in the plasma steroids (oestradiol, progesterone and testosterone) of female rhesus monkeys in the peri-ovulatory period. Changes in the females' access times were synchronised with the expected time of ovulation (solid horizontal bars) when the males' ejaculations were at their maximum. The 33 cycles were aligned on the day of the oestradiol peak (day 0). Vertical bars show standard errors of means. N , number of plasma samples; n , number of behaviour tests (five females, five males, nine pairs, 252 tests).

ovulation 24–48 h later¹⁰, may also synchronise and maximise the female's sexual motivation and the male's ejaculatory performance. The neuroendocrine mechanisms underlying the behavioural synchrony between the sexes and the timing of ovulation clearly have a selective advantage by optimising the chances for successful fertilisation and, thus, for survival in this highly evolved primate species in which sexual behaviour serves more than a purely reproductive function.

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Ca²⁺-dependent ATPase activity of bovine receptor cell outer segment

THE bleaching of as little as one molecule of rhodopsin can trigger hyperpolarisation of the vertebrate visual receptor cell. A light modulated Ca²⁺ flux may function in the receptor cell outer segments (OS) as an intracellular messenger amplifying and transducing the photochemical signal from its disk location to the external plasma membrane^{1,2}. In support of this hypothesis, Ca²⁺ has been shown to be concentrated in dark adapted disks³ and to be released on bleaching⁴⁻⁷. This suggests the presence of a Ca²⁺-dependent ATPase, an enzyme often associated with cellular Ca²⁺ pumps. The report that either the bleaching of a few per cent of the rhodopsin molecules within a frog rod suspension or the intra-OS elevation of Ca²⁺ to 10⁻⁷ M or greater, by means of ionophore A23187, can induce a rapid and very large decrease in ATP content⁸ provides indirect evidence for the presence of a Ca²⁺-activated ATPase. Interpretation of this finding is difficult, however, due to other known light-activated, ATP-dependent OS functions⁹. We present here direct evidence that purified bovine OS exhibit significant Ca²⁺-dependent ATPase activity, which seems to be independent of contributions from subcellular contamination.

Crude OS (OS_i) were prepared under dim red light from dark-adapted bovine retina (Hormel, Austin, Minnesota) by a sucrose floatation technique (modified from ref. 10), and highly purified OS (OS₂) were prepared by the method of Dratz¹¹. Both preparations were lyophilised and stored at -78 °C.

A Ca²⁺-dependent ATPase was present in the retinal homogenate and in OS_i. In contrast to the Mg²⁺-dependent and Na⁺, K⁺-enhanced Mg²⁺-dependent ATPases, each of which exhibit a threefold decrease in specific activity during OS_i purification, the specific activity of the Ca²⁺-dependent oligomycin-insensitive system increased (Tables 1 and 2) during purification.

A major difficulty in interpreting these results is that OS preparations are likely to contain mitochondria from the ellipsoid of the receptor cell¹², and mitochondria contain a Ca²⁺-dependent ATPase system¹³. Mitochondrial Ca²⁺-

dependent ATPase, however, is completely inhibited by oligomycin^{14,15}. Significantly, while oligomycin-sensitive, Ca²⁺-dependent ATPase activity is observed in the retinal homogenate, its specific activity decreases to a negligible amount as OS₂ are purified (Table 2). This suggests that mitochondrial contaminants contribute little activity, if any, to the total OS₂ Ca²⁺-dependent ATPase activity.

Table 2 Retinal Ca²⁺ dependent ATPase activity

Sample	10 µg oligomycin	No oligomycin
Retinal homogenate	5.9 ± 0.1(2)	8.8 ± 0.5(2)
Crude OS _i	6.8 ± 0.6(2)	7.5 ± 0.1(2)
Pure OS ₂	8.0 ± 0.7(1)	7.9 ± 0.8(1)

Method of assay was as described in Table 1. Oligomycin was introduced in 25% ethanol and samples were assayed in triplicate. Results are expressed in EU per mg protein as means ± s.e. with the number of assays in parentheses.

Maximal Ca²⁺ activation was observed at concentrations of 1 mM CaCl₂ or greater (Fig. 1), with no activity observed at concentrations less than 0.01 mM. Because the localisation of the ATPase and the availability of the added Ca²⁺ and ATP are unknown the Ca²⁺ concentrations could be considerably lower at the enzyme site. This might explain the high concentration required to activate the ATPase in these experiments compared with intracellular levels.

Localisation of the ATPase within the disk membrane would suggest a Ca²⁺ pump system which would account for the ability of the dark-adapted disk to accumulate Ca²⁺.

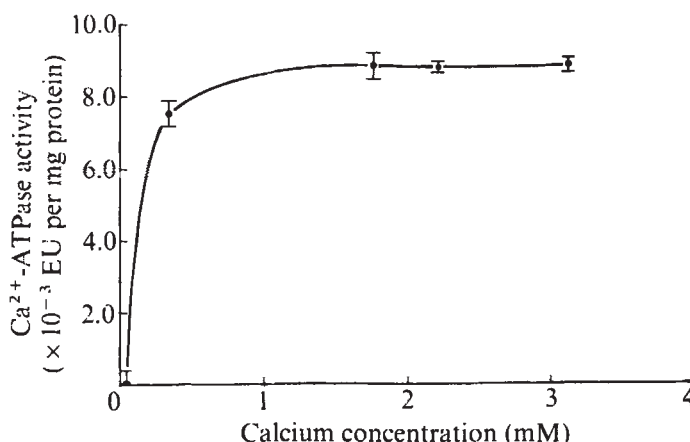


Fig. 1 ATPase activity as a function of free Ca²⁺. Ca²⁺-dependent ATPase was assayed as Table 1. CaCl₂ concentration varied. Free Ca²⁺ concentration was calculated using the association constant of the Ca EDTA²⁻ salt¹⁶.

Table 1 Retinal enzyme activities

Sample	Cyclic AMP diesterase	Mg ²⁺ ATPase	Na ⁺ K ⁺ ATPase	Ca ²⁺ ATPase
Retinal homogenate	7.4 ± 9(1)	29 ± 7(2)	8.8 ± 1.2(3)	5.9 ± 0.1(2)
Crude OS _i	30 ± 5(3)	9.9 ± 1.6(4)	3*	6.8 ± 0.6(2)

Cyclic nucleotide diesterase was assayed at V_{max}, using a modified two-step snake venom procedure¹⁶. Approximately 0.2 mg of protein was added to 1 ml containing 0.04 M Tris-HCl buffer pH 7.4, 0.09 M MgCl₂ and 0.02 M Tris cyclic AMP. The reaction was terminated after 4 min at 37 °C by a 1-min immersion in a boiling water bath. A second incubation at 37 °C with added *Crotalus atrox* venom yielded inorganic phosphate. In all assays phosphate was quantified colorimetrically by the method of Baginski *et al.*¹⁷. ATPase was assayed by the addition of 0.2-0.6 mg of protein to 1 ml solution containing one of the following solutions: 1, 0.1 mM EDTA, 3 mM MgCl₂, 0.05 M Tris-HCl buffer pH 7.4, 2 mM Tris-ATP, 1.35 mM Ouabain; 2, 0.1 mM EDTA, 0.15 M NaCl, 0.01 M KCl, 3 mM MgCl₂, 0.05 M Tris-HCl Buffer pH 7.4, 2 mM Tris-ATP; 3, 0.1 mM EDTA, 1 mM CaCl₂, 0.05 M Tris-HCl pH 7.4, 2 mM Tris-ATP, 10 µg oligomycin, 25% ethanol. After a 20-min incubation at 37 °C the reaction was terminated by the addition of 4 ml of a solution containing 9% trichloroacetic acid, 1.8% ascorbic acid and 0.015% EDTA. The samples were centrifuged and an aliquot of the supernatant was removed for phosphate determination. To correct for the co-precipitation of phosphate with protein, a control containing a phosphate standard was used. The experimental details of this procedure, developed by Dr Herman Kauffman and R.A.S., will be published elsewhere. Mg²⁺-dependent ATPase activity was obtained directly from the results of assay using mixture 1. Ouabain-sensitive (Na⁺ K⁺-enhanced Mg²⁺-dependent) ATPase activity was obtained by subtracting the Mg-dependent ATPase activity from that obtained with assay mixture 2. Assay mixture 3 yielded Ca²⁺-dependent, oligomycin-insensitive ATPase activity. Protein was determined by method of Lowry *et al.*¹⁸. All assays were carried out in triplicate, the number of separate assays being given in parentheses. Results are expressed in EU (µmol min⁻¹) per mg protein as means ± s.e..

*The specific activity of Na⁺ K⁺-enhanced, Mg²⁺-dependent ATPase in purified OS was low and the enzyme assay was unstable. Assays were linear for only 5 min and the results are approximate.

Plasma membrane localisation, which cannot be excluded, would suggest a Ca^{2+} -modulated energy utilisation system, possibly involved in hyperpolarisation.

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Ultraviolet light sensitivity and delayed DNA-chain maturation in Bloom's syndrome fibroblasts

BLOOM'S syndrome (BS) is a rare, autosomal recessive condition of pre-natal and post-natal growth retardation, sun-induced telangiectatic erythema, impaired immunological function and predisposition to cancer^{1,2}. BS cells show a high incidence of chromosome breakage and rearrangement often with abnormal nuclear morphology. Chromosome exchanges appear mainly to involve homologous chromosomes, while sister chromatid exchanges are abnormally frequent³. These features suggest a defect of DNA replication or repair. In particular, the high incidence of sister chromatid exchanges may be explained if free ends in the DNA, resulting from abnormalities of DNA synthesis and/or repair, act as initiation points for exchange⁴. We report here that BS fibroblasts are abnormally sensitive to ultraviolet irradiation *in vitro* and show a rate of DNA synthesis and DNA chain maturation lower than controls. They do not, however, have defective bypass of ultraviolet light-induced lesions during DNA replication as xeroderma pigmentosum (XP) variants⁵.

Skin fibroblasts from two patients with the typical clinical and cytogenetic features of BS (H.11325/PRU8638; H.12572/PRU10003), termed BS₁ and BS₂, respectively, and normal volunteers (H.9172; H.9527) were cultured from small skin biopsies in medium TC199, supplemented with 15% human serum

and 2.5% chick embryo extract and then stored in liquid nitrogen. Cells usually at the sixth to eighth and occasionally at the ninth to twelfth passage were used.

Typically, BS fibroblasts grow poorly and ours showed plating efficiency of 7–18%, while that of controls was 23–51%. The labelling index of BS cells incubated in the presence of ³H-thymidine (³H-Tdr) was lower than that of control cells (Table 1) and usually reached a lower plateau during a 3-d incubation (Table 2). This indicated that a larger fraction of cells was not replicating in BS than in normal cultures.

BS cultures were more sensitive to ultraviolet radiation than controls. Survival curves (Fig. 1), standardised for plating efficiency, show that the two BS strains were similarly affected and that their curves lack a shoulder. If sensitivity is expressed in terms of the dose resulting in 37% survival, the BS strains were three times as sensitive as controls.

In spite of the ultraviolet sensitivity of BS cells, our autoradiographic data (Table 3) suggest that exposure to ultraviolet light depresses DNA synthesis in control and BS cells by similar amounts. The average grain count of BS nuclei, however, was lower than that of controls (Tables 1 and 3), suggesting that the rate of overall DNA synthesis in BS nuclei is lower than in controls even in normal conditions.

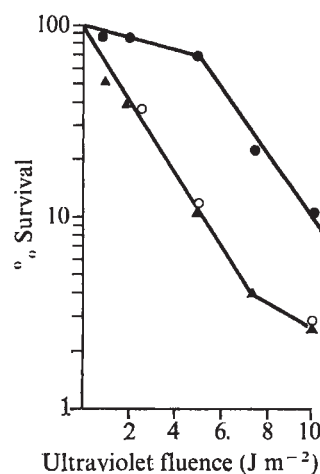


Fig. 1 Ultraviolet survival curves of normal (●) and BS (BS₁, ▲; BS₂, ○) fibroblasts. Inocula of 200 fibroblasts, plated in 60-mm plastic Petri dishes, were washed, after attachment, with phosphate-buffered saline and irradiated or sham-irradiated using a germicidal lamp emitting mainly ultraviolet light of 254 nm. When adequate colonies had grown in unirradiated cultures, all plates were collected and colonies of at least 50 cells were counted. The number of colonies scored at each ultraviolet dose is expressed as a percentage of the colonies in unirradiated cultures and shown on a logarithmic scale. The control curve was derived from eight experiments; that of BS₁ from four and that of BS₂ from one.

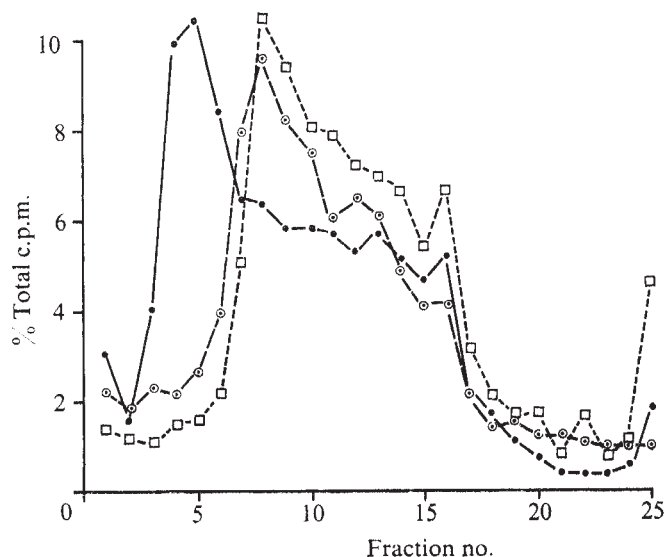
Pulse chase experiments showed that newly synthesised DNA from both BS cell strains consistently had lower molecular weights than DNA from control cells (Figs 2 and 3a). In time course experiments following a 1-h ³H-Tdr pulse, this difference was maximal after a chase of 2.5 h, but persisted with diminishing magnitude when the chase was extended for up to 6 h. Ultraviolet

Table 1 Percentage of ³H-Tdr-labelled cells and grain counts after 1-h pulse

Cell strain	1		2		Experiment no. 3		4		5	
	%LC	GC	%LC	GC	%LC	GC	%LC	GC	%LC	GC
BS ₁	5.8	40.5	5.4	41	3.2	57	16.7	2.6		
Control	45	92.3	25	93	15	110	38.4	18.5		

Coverslips from 60-mm Petri dishes, inoculated with 10⁵ cells at the sixth to ninth passage, were collected a day later after a 1-h pulse in ³H-Tr (12.5 Ci mmol⁻¹, 30 μCi ml⁻¹). They were then washed in phosphate-buffered saline (PBS), fixed in 3:1 methanol-acetic acid mixture and coated with AR 10 autoradiographic film. Autoradiographs were developed after 6-h exposure. Labelling indices (%LC) were estimated from samples of 2,000 cells and mean grain counts (GC) from 75 cells. BS cells show a 50% reduction in grain counts.

Fig. 2 Alkaline sucrose gradient profiles of newly-synthesised DNA in unirradiated control (●), BS₁ (□) and BS₂ (○) cells. 2.5×10^5 cells were cultured as described in the text in 60-mm Petri dishes and incubated for 2 d, washed in PBS (8 g of NaCl, 0.2 g of KH_2PO_4 , 0.2 g of KCl, 1 μg of Na_2HPO_4 per 1 of water) and incubated in 5 ml of warm medium for 1 h, pulse-labelled with ^3H -Tdr ($33 \mu\text{Ci ml}^{-1}$; 25°C mmol^{-1}) for 1 h, washed with warm PBS and chased for 2.5 h with unlabelled medium containing thymidine and deoxyeytidine (both at $10 \mu\text{M}$). After incubation, cells were scraped off the Petri dishes with a piece of silicone rubber into 0.3 ml of PBS containing EDTA (0.2%), centrifuged (600g for 5 min at 18°C) and resuspended in 0.15 ml of EDTA solution. The suspension was then exposed to δ irradiation (1.5–2.0 krad) from a ^{60}Co source to prevent entanglement of DNA strands⁶. Alkaline sucrose gradients (4.4 ml, 5–20%) containing 0.1 M NaOH and 0.1 M NaCl were overlain first with 0.2 ml of 2% sodium dodecyl sulphate containing 0.02 M EDTA; and then with 0.1 ml of the cell suspension. Centrifugation was for 75 min at 20°C and 40,000. p.m. in an SW56 rotor in a Beckman 1.2-65B ultracentrifuge. After centrifugation, 10-drop fractions were collected from the bottom of the tubes and assayed for cold acid-insoluble radioactivity⁷. Sedimentation was towards the left.



irradiation before labelling (Fig. 3b) depressed the maturation of DNA chains to a similar extent in BS and control cells, while caffeine had no detectable effect in unirradiated control or BS cells (Fig. 3c), but caused a moderate depression of DNA chain maturation in ultraviolet-irradiated control and BS cells (Fig. 3d). In contrast, and in agreement with Lehmann *et al.*⁵, cells from a patient with XP-variant showed a much greater inhibition of DNA chain maturation after ultraviolet irradiation and this was accentuated by caffeine (Fig. 4). This effect of ultraviolet irradiation on XP-variant cells has been attributed to a defect in the bypass of

induced lesions, probably due to a delay in the sealing of gaps left opposite pyrimidine dimers during the synthesis of new DNA strands (post-replication repair⁵). Ultraviolet irradiation with and without caffeine had no differential effect on the maturation of newly synthesised DNA of control and BS cells, suggesting that this form of repair is not inhibited in BS, as it is in XP-variants.

Our experiments show that BS fibroblasts *in vitro* have low viability and are abnormally sensitive to ultraviolet irradiation. Their rate of DNA synthesis is low and maturation of new DNA chains is delayed. This is in keeping with the clinical features of BS,

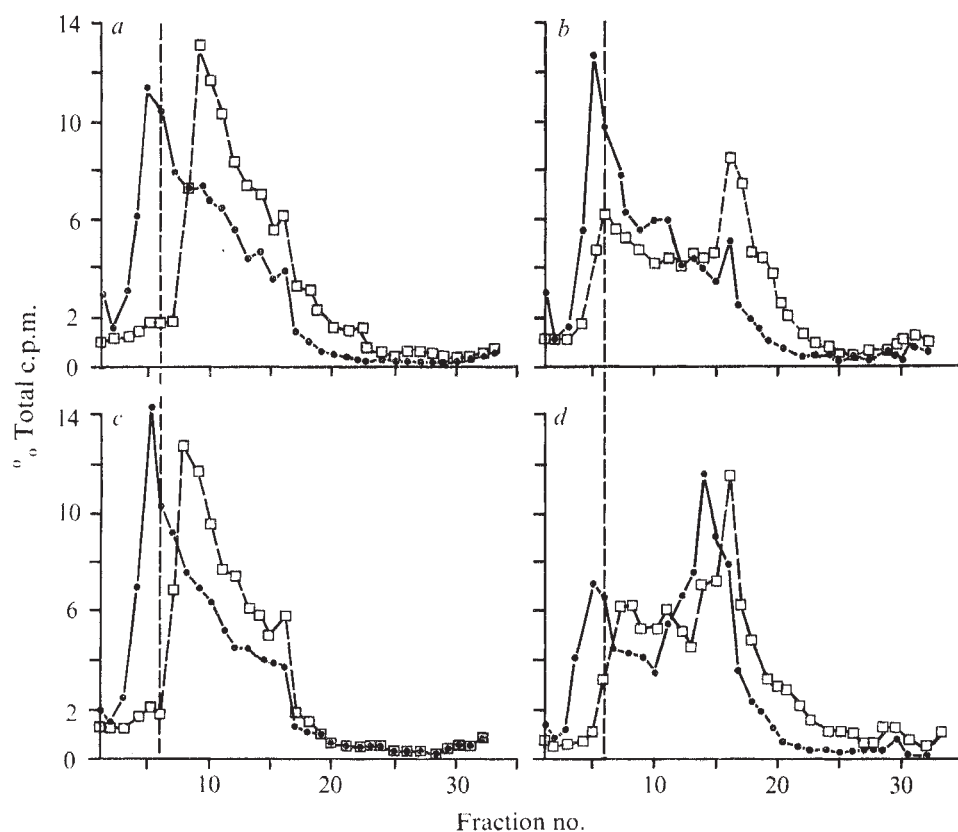


Fig. 3 Alkaline sucrose gradient profiles of DNA newly synthesised, either in the absence or presence of caffeine, by ultraviolet-irradiated and non-irradiated BS₁ (□) and control (●) cells; methods were as described in Fig. 2, except for irradiated and/or caffeine-treated cells which were exposed, immediately after the initial washing with PBS, to germicidal light (mainly of 254 nm) at a fluence of 12.5 J m^{-2} and/or caffeine (0.3 mg ml^{-1}). The vertical line marks the sixth fraction of each gradient. *a*, Nonirradiated, no caffeine; *b*, Irradiated, no caffeine; *c*, non-irradiated caffeine; *d*, irradiated, caffeine. As in Fig. 2, the DNA of the BS cells sediments more slowly than the control and this difference is not influenced by the treatment, even where irradiation and caffeine result in a noticeable reduction in the sedimentation rate of both control and BS DNA (*d*).

Table 2 Percentage of ^3H -Tdr-labelled fibroblasts as a function of time

Cell strain	Experiment no.	Labelling period (h)					
		14	24	38	48	62	72
BS ₁	1*	9.25	19.4	—	29.7	31.7	—
	2†	33.1	34.0	35.6	41.1	38.9	53.4
BS ₂	2	55.5	69.5	74.0	74.8	75.6	78.5
	3‡	10.7	35.5	—	44.0	55.0	59.0
Control	1*	46.9	89.2	—	92.0	93.6	—
	2	59.1	66.5	72.1	71.4	69.4	74.1
	3	30.4	76	—	72	78	83

*Cells at the eight passage.

†Cells at the fifth passage.

‡Cells at the ninth passage.

Cells at similar passages were plated in 35-mm Petri dishes containing coverslips (4×10^4 per dish) and labelled with ^3H -Tdr ($1 \mu\text{Ci ml}^{-1}$, 1 Ci mmol^{-1}). The label was replenished daily. Collected coverslips were washed with PBS, fixed in 3:1 methanol-acetic mixture and coated with AR 10 autoradiographic film. Autoradiographs were developed after 10-d exposure. Samples of 2,000 cells were used to determine labelling indices.

Table 3 Mean grain counts per labelled nucleus after 1-h ^3H -Tdr pulse

Cell strain	Experimental treatment							
	No irradiation, no caffeine		No irradiation, plus caffeine		Irradiation, no caffeine		Irradiation, plus caffeine	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
BS ₁	119	6.3	127	8.8	39.7	2.4	50.1	2.8
Control	131	8.1	182	8.4	71.0	4.0	76.9	3.9
BS ₂	125	8.1	—	—	41.5	4.2	42.7	4.7
Control	186	7.6	227	23.4	58.3	2.6	62.6	3.6

Cells were plated in Petri dishes containing coverslips and treated as for those in Fig. 3. They were collected and autoradiographs were prepared as described in Table 1. Samples of 50 cells were used to calculate the mean grain counts. BS cells show a 30% reduction in grain counts. This is less than observed in Table 1; such discrepancy may be due to the different experimental procedures to which the cells have been exposed.

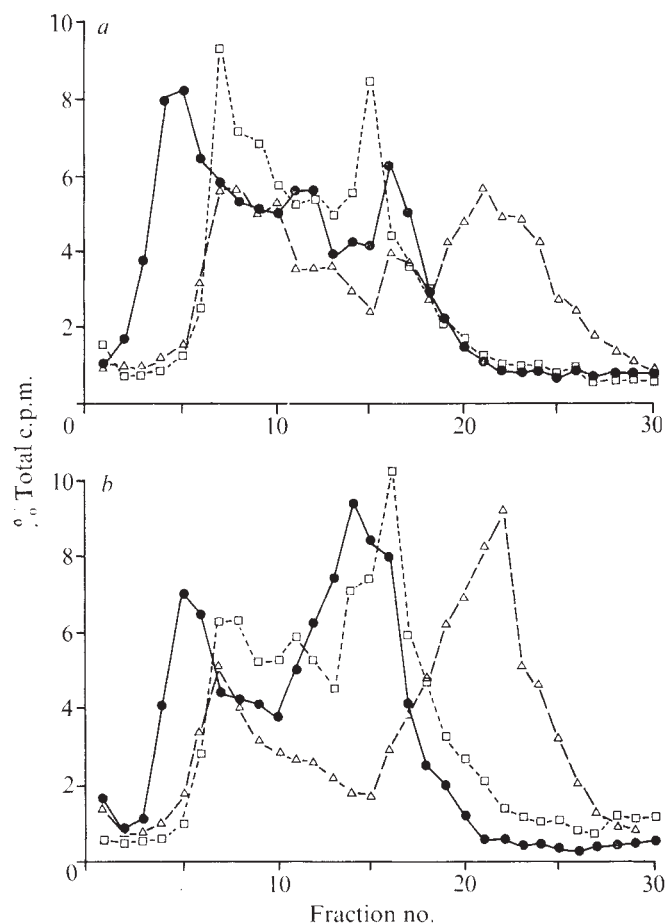


Fig. 4 Alkaline sucrose gradient profiles of DNA newly synthesised, either in the absence or presence of caffeine by ultraviolet-irradiated control (●), BS (□) and XP variant cells (△) labelled and chased as in Figs 2 and 3. Experiments were carried out either in the absence (a) or presence (b) of caffeine (0.3 mg ml^{-1}), throughout the period after ultraviolet irradiation.

particularly growth retardation and light sensitivity. In contrast to XP-variants, when BS cells are exposed to 12.5 J m^{-2} of germicidal ultraviolet light they do not seem to have difficulties in bypassing ultraviolet-induced lesions in the course of DNA replication and they seem to respond normally to the inhibitory action of ultraviolet light on DNA synthesis.

Data of Hand and German⁸ suggest that movement of DNA replicating forks is slower in BS than in control cells. By their autoradiographic technique, they could measure DNA chain growth of relatively small samples of replicons from a few replicating cells. Our sedimentation experiments, however, compare the average rate of maturation of DNA chains attaining a maximum size of $150 \mu\text{m}$ (approximately 5 replicons $30 \mu\text{m}$ long) from whole cell populations^{5,9}.

Our results therefore are in line with and extend those of Hand and German⁸. Because their technique depends on cellular uptake of ^3H -Tdr, they considered the possibility that their results reflect impaired uptake of ^3H -Tdr by BS fibroblasts. We excluded this possibility because, after a 2.5-h chase (during which DNA synthesis was not dependent on exogenous ^3H -Tdr), differences in molecular weight of BS and control DNA were even greater than immediately after the pulse (data not shown).

DNA synthesis in mammalian cells is initiated in a pre-programmed fashion at different sites of the same molecule and proceeds bilaterally from each origin⁹⁻¹¹. The rate of movement of the growing fork may change during the S phase¹⁰ and the distance between initiation points may vary between cells of the same organism according to the stage of differentiation and development¹¹. Our data for BS cells suggest that the fundamental defect involves a step in DNA replication, but we cannot exclude the possibility that the impaired growth of new DNA chains is secondary to a disturbance of other metabolic processes. The abnormal sensitivity of BS fibroblasts to ultraviolet light cannot yet be explained by a defect of DNA repair. For analysis of the first steps of excision repair has given negative results^{12,13} and our observations suggest that the bypass of ultraviolet lesions is not impaired as in XP-variants. The association of ultraviolet-sensitivity and delayed maturation of DNA chains in BS fibroblasts, however, suggests that the metabolic defect in BS involves a

factor or step important for DNA synthesis and for the repair of ultraviolet-induced damage.

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Chromatin dispersal and DNA synthesis in G1 and G2 HeLa cell nuclei injected into *Xenopus* eggs

If HeLa cells that are synthesising DNA (S phase) are fused with others in the G1 phase of the cell cycle, DNA synthesis is prematurely induced in the G1 nuclei. But if S-phase cells are fused with G2 phase cells, which have already replicated their DNA, synthesis is not induced in the G2 nuclei, although it continues in the S-phase nuclei¹. Evidently nuclei of G2 cells contain components that prevent replication of their DNA even in the presence of the enzymes and substrates. If cell nuclei are injected into eggs of *Xenopus laevis* they usually swell within 1 h to many times their original volume, the chromatin becoming dispersed throughout the swollen nucleus. Simultaneously, DNA replication begins in the nuclei². We have injected nuclei of G1 and G2 HeLa cells into *Xenopus* eggs in a study of the relationship between this chromatin dispersal and DNA synthesis, and the restriction to DNA synthesis in G2 nuclei.

Figure 1 shows the size distribution of G1 and G2 HeLa cell nuclei 5, 60 and 120 min after injection of ruptured HeLa cells into *Xenopus* eggs. At 5 min, before the nuclei had swollen appreciably, they were all below 17- μ m diameter. Also, the distribution of G1 and G2 nuclei between the two size groups was roughly the same (although a small but significantly higher percentage of G2 nuclei than G1 were greater than 12- μ m diameter ($P=0.05\%$)). By 60 min some of the G1 and G2 nuclei had swollen above 17 μ m, and by 120 min many more had done so and also many of the smallest nuclei had moved into the middle size group. At 120 min any differences in size distribution between G1 and G2 nuclei were of little significance ($P>20\%$). Hence G1 and G2 HeLa cell nuclei swell to a similar extent in *Xenopus* egg cytoplasm.

Xenopus egg cytoplasm, however, does not induce similar rates of DNA synthesis in the nuclei of these ruptured G1 and G2 HeLa cells. Figure 2 shows the number of silver grains over sections of HeLa nuclei in *Xenopus* eggs which were fixed for autoradiography 60 min after injecting ruptured G1 and G2 HeLa cells and ³H-thymidine. The number of grains over the most swollen G1 nuclei was comparable with that counted over the pronuclei of the

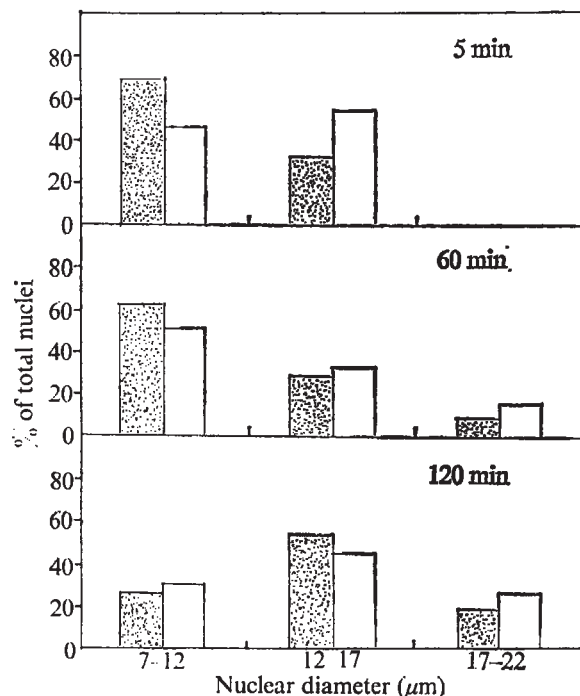


Fig. 1 Size distribution of HeLa cell nuclei at intervals after injection of ruptured HeLa cells into *Xenopus* eggs. G1, stippled; G2, plain. HeLa cells were grown as monolayers in Eagle's medium with added foetal calf serum, and synchronised by double thymidine block³. Cells were suspended for injection in a solution of sucrose (1.2 M), MgCl₂ (1.5 mM), vinblastine sulphate (0.625 mM) and Tris-HCl (1 mM) pH 7.2. *X. laevis* females were induced to ovulate¹, and the eggs dejellied². About 20 cells were injected⁴ into each egg in 50 nl of medium which also contained ³H-thymidine (25 nCi, 0.4 ng Radiochemical Centre, Amersham). Vinblastine was present to retain the egg pronucleus at the animal pole. After 5, 60 or 120 min at 18 °C the eggs were fixed in a modified Perenyi's fluid and sectioned at 5 μ m. Sections were mounted and subjected to autoradiography with Nuclear Emulsion L 4 (Ilford) with 7-d exposure at 0-2 °C. They were then stained with Mayer's haematoxylin and eosin. Nuclear diameters of over 100 G1 and G2 nuclei in over 20 eggs were measured at each time with a calibrated eyepiece graticule as the mean of the longest and shortest axis.

Xenopus eggs, namely a mean of 26.6 over 10 pronuclei. Precise comparisons from these counts of rates of DNA synthesis between the HeLa cell nuclei and *Xenopus* pronuclei are impossible because of the different concentration of DNA per unit area of cross-section. But, because the egg pronuclei completely replicate their DNA within about 45 min of activation of the egg by pricking², it is clear that the largest G1 nuclei must also have replicated a large part of their DNA. As the size of the G1 nuclei decreases, the number of silver grains per nuclear section also decreases (and hence the ³H-thymidine incorporated per nucleus must decrease more rapidly still, since the sections of smaller diameter contain a higher proportion of the nuclear DNA).

In contrast, all G2 nuclei incorporated little thymidine, so the block to DNA replication of even the most swollen G2 nuclei has largely been retained, and is effective against the replicating enzymes of the *Xenopus* egg.

In these experiments ruptured whole HeLa cells were injected into the eggs. Figure 3 shows the results of an experiment which was identical except that before injection the G1 and G2 HeLa cell nuclei were isolated by a standard procedure. The G1 nuclei incorporated roughly the same amounts of ³H-thymidine as before, but the G2 nuclei incorporated almost as much as the G1, showing that their block to DNA replication has been lost.

DNA replication in the egg and sperm pronuclei after fertilisation is always accompanied by swelling of the pronuclei⁵, and previous work has suggested that there is a causal connection between the two processes. Thus, when

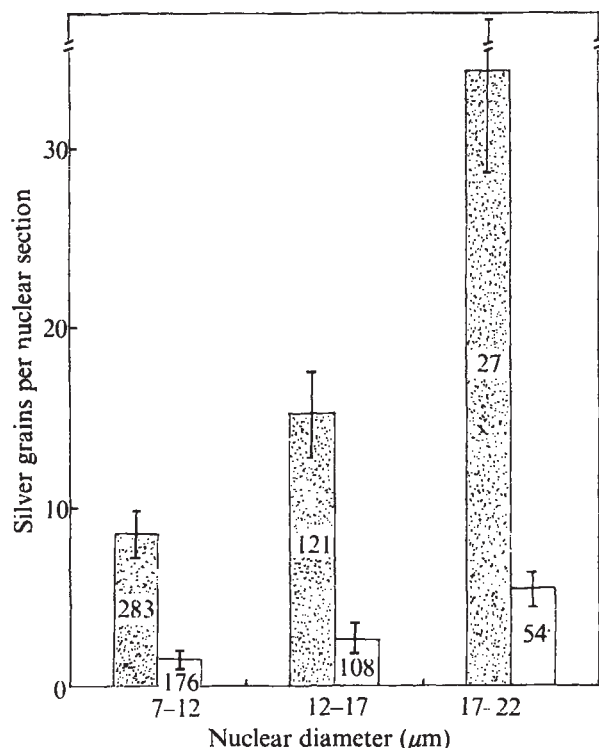


Fig. 2 ^3H -thymidine incorporated into nuclei of ruptured HeLa cells by 60 min after injection into *Xenopus* eggs. G1, stippled; G2, plain. The experiment was performed as described in Fig. 1. The number of silver grains per nucleus is the number over each nucleus less the number over a similar area of egg cytoplasm in the same section, values are mean $\pm$ s.e. The number of nuclei scored is shown in each column.

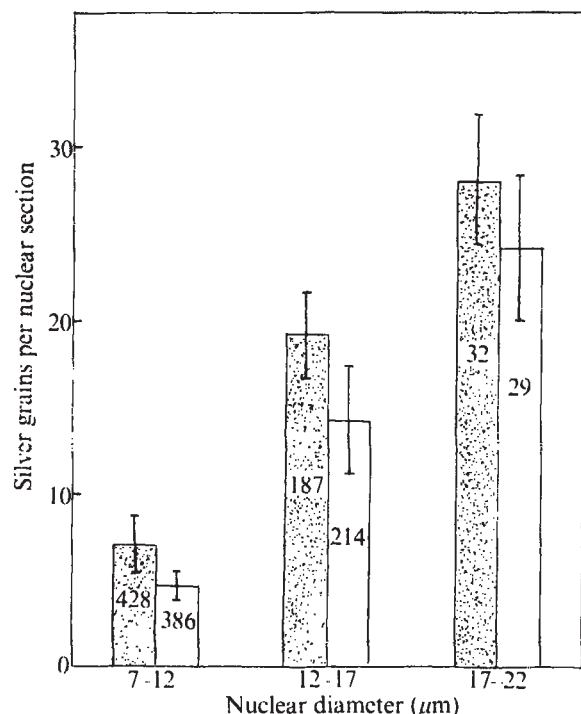


Fig. 3 ^3H -thymidine incorporated into isolated HeLa cell nuclei by 60 min after their injection into *Xenopus* eggs. G1, stippled; G2, plain. HeLa cells were suspended in a solution of MgCl_2 (3 mM) and Triton-X100 (0.1%) and, after 15 min, homogenised in a glass tube with Teflon plunger. The homogenate was centrifuged for 10 min, at 900g, resuspended in sucrose (0.32 M) and MgCl_2 (3 mM) and centrifuged again. The pellet was suspended in a little sucrose (2.1 M) and MgCl_2 (1.5 mM), layered on top of the same solution, and centrifuged for 1 h at 100,000g. The nuclei were suspended for injection as described in Figs 1 and 2. Data are mean $\pm$ s.e.

somatic cell nuclei were injected into *Xenopus* eggs, nuclei that did not incorporate ^3H -thymidine were little if at all swollen, and the average volume of the nuclei increased at the same rate as the proportion that replicated DNA². Also, those parts of injected brain nuclei which were the first to swell were the first to synthesise DNA⁸. It has not been clearly demonstrated, however, whether swelling is an essential prerequisite for DNA replication, or merely a consequence of it. Our finding that when ruptured HeLa cells are injected into *Xenopus* eggs G1 and G2 swell to the same extent, but only G1 nuclei replicate a significant portion of their DNA, strongly suggests that swelling is not merely a consequence of DNA synthesis, but is a prerequisite for it. Nuclear swelling in the *Xenopus* egg, with its associated chromatin dispersal, seems to be dependent on a lowering of the unbound cation concentration of the egg cytoplasm to a level at which bound cations dissociate from the DNA of chromatin^{4,9}, but seems to be independent of the discharge of cortical granules at activation¹⁰.

The ability to inhibit DNA replication is thus retained in the nuclei of ruptured G2 HeLa cells injected into *Xenopus* eggs, but almost completely lost if the nuclei are isolated before injection. Hence the inhibition is most probably caused by a loosely bound constituent not normally reckoned among the components of nuclei and chromatin, or by a labile modifying group attached to a known component. Alternative methods of isolating G2 HeLa cell nuclei may retain the ability to block DNA synthesis and facilitate identification of the components involved.

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Evidence for cytoplasmic control of gene expression in higher plants

ONE of the difficulties of studying regulatory gene function in higher eukaryotes is the lack of genotypes with single gene differences in constant genetic backgrounds. In the Triticinae, however, the construction of a series of aneuploids^{1,2} makes it possible to monitor the activity of single gene loci in different genetic backgrounds. With these genotypes we have been able (unpublished results with T. Lelley) to localise the structural genes for phosphodiesterase (PDE, EC 3.1.4.1.) on chromosomes 3A, 3B and 3D of wheat, *Triticum aestivum* ($2n=6x=42$). We also showed that regulatory genes located on chromosomes 5A, 5B and 5D enhance the activity of the structural genes. We now report that extrachromosomal control of the PDE isoenzymes is another component of this complex regulatory system.

Reciprocal hybrids were constructed between hexaploid wheat and its diploid relative, *Secale cereale* ($2n=14$), resulting in amphidiploid derivatives with wheat cytoplasm in

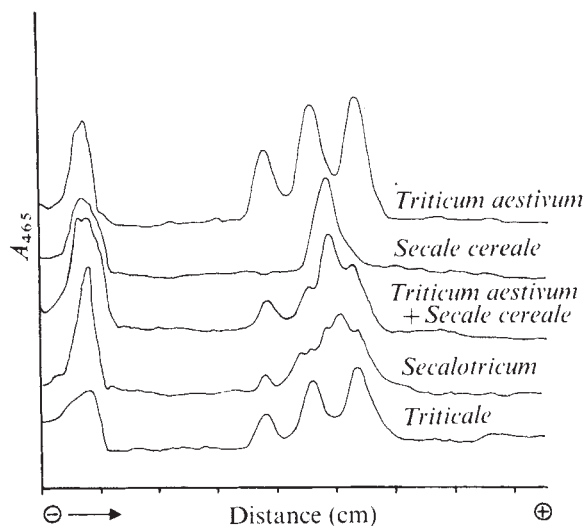


Fig. 1 Densitometer tracings of PDE isoenzyme patterns of wheat, rye, and wheat/rye hybrids. Extraction from leaves with $3 \times (w/v)$ 0.05 M Tris-HCl, pH 7.5, at 4 °C. Centrifugation was at 20,000 g for 10 min. This crude extract was used for separation in 7% polyacrylamide gels, 0.6×9 cm, according to Davis⁹. The gels were stained for 5'-phosphodiesterase by incubation in 0.05 M Tris-HCl, pH 7.5, and 2'-deoxythymidine-5'-phosphoric acid-1-naphthylester and Fast Blue RR for 30–60 min. The gels were scanned in a Joyce-Loebl densitometer at 465 nm. (The peak in the upper part of the gels is caused by an unspecific adsorption of Fast Blue to fraction-I protein).

Triticale ($2n=8x=56$) and with rye cytoplasm in *Secalotriticum* ($2n=8x=56$). We used several individual plants per genotype with cytologically verified chromosome numbers.

Polyacrylamide gel electrophoresis patterns of the PDE isoenzymes of both genotypes were compared with those of the parents (Fig. 1). The wheat pattern showed the well known three isoenzymes^{4,11}. The slow-moving enzyme is a product of the gene on chromosome 3D (*Pde-D3*); two fast-moving isoenzymes with an identical mobility are produced by the structural genes *Pde-A3* and *Pde-B3* on chromosomes 3A and 3B, respectively, and the hybrid enzyme is composed of the product subunits of the genes *Pde-D3*, *Pde-A3* and *Pde-B3*. Rye possesses only one PDE enzyme. Its mobility was identified by coelectrophoresis of wheat and rye extracts (Fig. 1) as being between those of the fast moving isoenzyme and hybrid enzyme of wheat. The *Secalotriticum* pattern clearly shows the wheat and rye isoenzymes while the pattern of the *Triticale* with wheat cytoplasm does not exhibit the rye band. Thus the rye gene is expressed in the presence of rye cytoplasmic factors but not in the *Triticale* with wheat cytoplasm. Two additional isoenzymes in *Secalotriticum* were not present in the

parent species. The number, positions and dimeric nature of the PDE isoenzymes (unpublished work of G. W.) makes them likely to be hybrid enzymes composed of wheat and rye monomers (Fig. 2). Further evidence for the cytoplasm-dependent expression of the rye PDE gene came from wheat cytoplasmic wheat rye addition lines³, where the seven rye chromosomes were added separately to the wheat genome. No difference in PDE activity was found between any of the seven addition lines and wheat or *Triticale* (not shown). Thus it is clear that the structural gene for the rye PDE enzyme is under cytoplasmic control. This gene cannot be expressed in genotypes with wheat cytoplasm. The structural and regulatory genes of wheat, however, are active in the presence of the rye cytoplasm.

In summary our studies on the genetic control of the PDE isoenzymes in the Triticinae reveal a complex system of regulation of gene expression. Our earlier studies (unpublished) showed that (1) the activity of the isoenzymes depends on the dose of the structural genes; (2) the activity of the structural genes is regulated by separate nuclear genes; (3) the effect of the regulatory units is also dose-dependent; (4) the regulatory and structural units are located on different genomes (B and D) of the allohexaploid wheat; thus, the complexity of regulation seemed to have increased with polyploidisation. We have now shown further that (5) in rye corresponding structural genes for PDE activity are under cytoplasmic control.

There have been several reports of the location of structural genes coding for enzyme proteins in wheat^{5–7}. Also, cytoplasmic control of the expression of nuclear genes has been observed in cereals⁸. The effects described, however, were only quantitative and reflect a type of gene control which is well documented. Our study also demonstrates qualitative effects—the absence of isoenzymes—of cytoplasmic factors on the expression of a nuclear gene with a well defined product. The PDE isoenzymes of wheat and rye are sufficiently homologous to form hybrid enzymes (Fig. 1), and so it is surprising that the corresponding regulatory factors of the wheat and rye cytoplasm are apparently quite different in their function. The fact that the nuclear structural genes of wheat are expressed in the presence of the rye cytoplasm may be a consequence of the polyploid nature of wheat where enhancing regulatory genes are present in triplicate (for example, on chromosomes 5A, 5B and 5D, unpublished results with T. Lelely). Our results strongly suggest that there is a complex interaction of structural genes not only with nuclear regulatory factors but also with cytoplasmic control factors, which are inherited from the female parent only. We therefore suggest that future experiments on gene regulation should take into account the existence of (as in this case) strong extrachromosomal control factors, so that existing models on regulation of gene expression (see, for example, refs 10 and 11) may be extended. Our hypothesis of gene regulation can be tested further using haploid hybrids, rye cytoplasmic wheat and rye cytoplasmic addition lines which are now being constructed.

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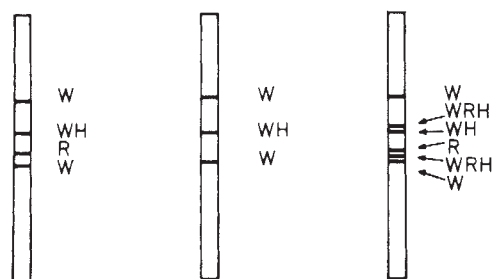


Fig. 2 Diagrammatic interpretation of the PDE isoenzyme patterns in wheat/rye hybrids. W, wheat enzymes; WH, wheat 'hybrid' enzymes; R, rye enzyme; WRH, wheat/rye 'hybrid' enzymes.

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High resolution detection of DNA-RNA hybrids *in situ* by indirect immunofluorescence

We describe here a new method for the detection of RNA-DNA hybrids in cytological preparations with which we have revealed the locations of hybrid molecules on polytene chromosomes. The critical reagent is an antiserum raised in rabbits against poly(rA)-poly(dT) complexed with methylated bovine serum albumin, originally described by Stollar¹. The specificity and resolving power of the indirect immunofluorescence procedure are demonstrated using *in situ* hybridisation of 5S rRNA (ribosomal RNA) to polytene chromosomes of *Drosophila melanogaster* as a model system. The method has significant advantages over the autoradiographic procedures²⁻⁵ used so far.

The procedure for visualising the *in situ* hybrids follows Alfageme *et al.*⁶ It consists of exposing the cytological preparation to the rabbit anti-hybrid antiserum, then to anti-rabbit IgG prepared in goat and tagged with rhodamine, followed by examination in a fluorescence microscope (see legend to Fig. 1). Our test objects were polytene chromosomes of *Drosophila melanogaster* (giant phenotype) to which 5S rRNA had been hybridised *in situ* (see legend to Fig. 1). The two glands from a single larva either were used as duplicate samples or one gland served as a 'control' for the other. The preparations were not air dried at any time during the procedures. Test slides which were air dried at some stage before the immunological reactions were inferior to their controls either in the morphology of the chromosomes, the background fluorescence levels, the uniformity of the fluorescence staining or a combination of defects.

In situ hybridisation followed, in general, the recipe of Pardue and Gall⁷ with modification by Alonzo *et al.*⁸ (see legend to Fig. 1). The reaction was effective with 1.0 µg and with 0.2 µg 5S rRNA per slide. It is likely that much smaller amounts could be used if applied in a smaller volume and, perhaps, for longer times⁹⁻¹¹.

The specificity of the immunological reagents in the cytological reaction is demonstrated by the confinement of chromosomal fluorescent label to the 56F region when 5S RNA is included in the *in situ* hybridisation medium (Fig. 1) and by the absence of chromosomal fluorescence when 5S rRNA is omitted (not shown; would be black). Further evidence that the anti-hybrid antibodies are responsible for the chromosomal site of the positive fluorescence reaction is provided by the absence of fluorescence in chromosomes to which 5S RNA had been hybridised but which were treated with antiserum absorbed with poly(rA)-poly(dT) (5 µg poly(rA)-poly(dT) per µl serum for 24 h at 4 °C, centrifuged for 10 min at 6,000g). Thus the immunological reagents revealed only DNA-RNA hybrids within the nuclei of polytene cells prepared for *in situ* hybridisation.

The specificity was demonstrated further by analyses of the immunological properties of the antiserum. As described previously, several-thousand-fold dilutions of serum reacted in complement fixation assays with poly(rA)-poly(dT), poly(I)-poly(dC) or hybrids of natural RNA and DNA (ref. 1). A 1/50 serum dilution did not react with any single-stranded form of RNA or DNA or with double-stranded RNA or native DNA. When serum was assayed undiluted in counterimmunoelectrophoresis, weak reactions were seen with poly(rA) and with denatured DNA. Both these reactions were eliminated when the serum was passed through a poly(rA)-Sepharose affinity column prepared as described by Poonian *et al.*¹⁰ (Fig. 3). With absorbed serum, which gave the same immunofluorescence as unabsorbed

serum, the hybrid was the only reactive polynucleotide class, even in assays with undiluted serum.

There is a variable amount of fluorescence in cytoplasmic components, the origin of which is not yet known. Experiments are projected to attempt to block it while leaving the activity against hybrid nucleic acids intact. Occasional pale fluorescence observed in nucleoli is attributed to contamination of the 5S rRNA probe with fragments of 18S and 28S nucleolar rRNA,



Fig. 1 5S rRNA genes revealed in polytene chromosome 2R of *Drosophila melanogaster* by indirect immunofluorescence detection of RNA-DNA hybrids formed *in situ*. The two homologous 2R chromosomes are not paired except in their most distal portions. The 5S genes (56F on Bridges' standard map) are in the unpaired portions, those derived from one parent to the right, the other parent to the left. In each homologue at least two fluorescent cross-bands are visible. Arrows indicate the 56F regions in the upper photograph of the same chromosomes taken after staining with aceto-orcein. A salivary gland from a fully grown larva of *D. melanogaster* (giant phenotype) was fixed in 50% (v/v) aqueous acetic acid and squashed under a siliconed cover glass, then the slide was frozen on solid CO₂. After snapping off the cover slip, the slide was post-fixed in 3:1 ethanol-acetic acid (v/v), rinsed twice in 95% aqueous ethanol (v/v) and stored in 95% ethanol until used. Subsequent treatments were carried out in a moist chamber consisting of a 90-mm square culture dish containing a few leaves of bibulous (or filter) paper saturated with the solvent and two plastic strips to raise the slides above the wet paper. The reagent was placed between the slide and a cover slip. For hybridisation *in situ*, slides were first treated with pancreatic ribonuclease, 100 µg ml⁻¹ of 2 × SSC (SSC is 0.15 M NaCl and 0.015 M sodium citrate) for 2 h at 25 °C, then with 90% formamide in 0.1 × SSC for 2 h at 65 °C followed by ice cold 0.1 × SSC rinses. The hybridisation reaction was carried out for 22-24 h at 37 °C in 50% formamide in 4 × SSC using either 1.0 or 0.2 µg 5S RNA per slide—highly purified 5S rRNA, extracted from *D. melanogaster* Oregon R embryos¹¹. After the annealing reaction, the slides were treated with pancreatic ribonuclease (15 µg ml⁻¹) in 2 × SSC for 2 h at 25 °C, rinsed with phosphate-buffered saline (PBS, 0.14 M NaCl, 0.01 M phosphate, pH 7.2) and exposed for 2 h at room temperature (21-23 °C) to rabbit anti-DNA-RNA hybrid serum reconstituted from a lyophilised state by solution in water and diluted for use in PBS (1:20 for Fig. 1). After thorough rinsing in PBS, the slides were finally exposed to a rhodamine-labelled goat IgG fraction of anti-rabbit-IgG (Miles-Yeda) reconstituted to approximately its original concentration, then diluted in PBS for use (1:20 for Fig. 1). The photographs were taken with a microscope equipped with a Zeiss epi-illumination fluorescence module using a 546-nm excitation filter, a 580-nm chromatic splitter and a 580-nm barrier filter for rhodamine fluorescence on 35-mm Eastman Tri X Pan film exposed at ASA 1600 (Difine developer) at magnifications of approximately ×150 (×40 objective) or ×370 (×100 oil immersion objective). Fluorescence exposures were in the range of 1-8 s and phase contrast illumination was adjusted to require approximately the same exposure time so that a single frame could be exposed in both modes simultaneously (not shown here). In some instances, slides were stained with aceto-orcein after fluorescence photography had been completed, then photographed through a No. 58 filter (green) on Plus-X-Pan film at ASA 400. Approx. ×1,000.

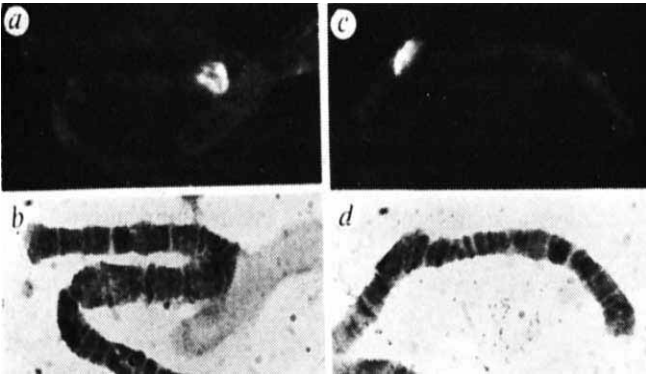


Fig. 2 5S rRNA genes revealed in *D. melanogaster* polytene chromosome 2R as in the legend to Fig. 1. The two chromosomes are unpaired in a single nucleus. The upper chromosome (*a* and *b*) is associated with the nucleolus (nu) at the locus of the 5S genes, fluorescent in (*a*). The homologous chromosome (*c* and *d*) is not visibly associated with the nucleolus; its 5S locus, indicated by the fluorescence in (*c*), is less dispersed than that in its homologue (*a*). (*b* and *d*) Photomicrographs of the same fields as in (*a*) and (*c*), respectively, after staining with aceto-orcein. $\times 630$.

but the possibility that 5S DNA templates occur in nucleoli cannot be excluded.

The spatial resolving power of the immunofluorescent probe is equal to that of the optical system used to observe it. Autoradiographs can at best reveal a cluster of silver grains adjacent to, or covering, a labelled region which results in a resolving power of the order of 1–3 μm . So far the precise localisation of the 5S genes within the 56EF region has been equivocal for *D. melanogaster* using autoradiography^{1,9}. The images we obtain are of two kinds. In some nuclei, the fluorescence is restricted to a relatively narrow transverse 'band' which is often clearly made up of two subunits and is localised in the 56F region, distal to the puff usually present in 56E (Fig. 1). The possibility that the two subunits reflect the organisation of the 5S locus into the two separable sets of repeated sequences recently reported by Procunier and Tartof¹² is under investigation. On the other hand, the 5S region of chromosome 2R sometimes sticks to the nucleolus as in Fig. 2 (ref. 9) or is ectopically paired

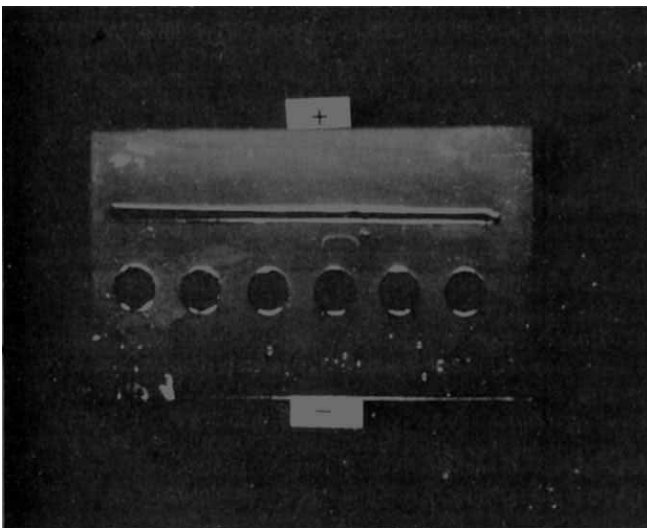


Fig. 3 Specificity of the absorbed antiserum. A sample of 7 ml of anti-poly(rA)·poly(dT) antiserum was absorbed by passage through a 1.2-ml column of poly(rA)–Sepharose equilibrated with 0.1 M NaCl, 0.01 M phosphate, pH 7.2. The undiluted absorbed serum (200 μl) was placed in the trough; the wells contained 0.5 μg polynucleotide in 50 μl running buffer (0.05 M Tris-HCl, pH 8). The polynucleotides were, from left to right: poly(rA); denatured DNA; poly(rA)·poly(dT); poly(dT); poly(l)·poly(C); and poly(dA). The gel medium was 0.8% agar (Difco, purified agar) in running buffer; 7 ml of gel was poured on each 2 $\times$ 3 inch glass plate. Electrophoresis was run at 220 V (10 m per plate) for 45 min.

to other chromosome regions. In those cases, the fluorescence may be distributed in a network of fibrils extending longitudinally along a much longer segment of chromosome (Fig. 2*a*). When that is true, the morphology in phase contrast and/or after post-staining with aceto-orcein is atypical in that the subsections 56E and F cannot be clearly demarcated and the region does not appear to be organised into distinct bands. Such dispersion of the *in situ* hybrids into fibrils is consistent with the suggestion of Steffensen and Wimber⁹ that the 5S genes may have been active in those chromosomes¹³. But the possibility that our 5S probe contains traces of contaminating nucleolar rRNA fragments that could reveal nucleolar rDNA adhering to chromosome 2 has not been entirely excluded.

The sensitivity of the technique has not yet been fully explored. At serum dilutions of 1 : 40 for the anti-hybrid rabbit serum and an equivalent concentration for the fluorescent reagent, the fluorescence intensity in the 5S region was very high. Photographic images should still be easily recordable at brightnesses one to two orders of magnitude lower. Thus, the possibility to detect the hybridisation of RNA copies of a unique gene in a polytene chromosome appears to be real. On the other hand, genes present in a size and multiplicity equivalent to the 5S of *D. melanogaster* may be detectable in unimicrometres chromosomes. An attempt is in progress to detect the 5S locus in human chromosomes at the pachytene stage of meiosis.

The technique is being used to study the distribution of naturally occurring chromosomal RNA detected as hybrid molecules. Polytene chromosomes mounted out of 50% acetic acid display a pattern of fluorescent regions when treated only with the immunological reagents. The regions do not fluoresce if the anti-hybrid serum is blocked with poly(rA)·poly(dT) or if the chromosomes are treated to remove indigenous chromosomal RNA (as in preparation for *in situ* hybridisation), indicating that they are sites of hybrid molecules. Since the locations of the sites change during larval and prepupal development, their RNA moiety could be involved with the control of transcription or of replication or both.

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Anion modulation of the negative Bohr effect of haemoglobin from a primitive amphibian

THE Congo eel (*Amphiuma means*) is a primitive salamander that respire through its lungs, gills and integument. It lives in swamps, ditches and rice fields in the south-eastern USA. When pools and swamps become stagnant and water levels drop, *Amphiuma* will burrow and hibernate in the mud. In this letter we report that when organic phosphate cofactors are low or absent, as might be true during hibernation, the haemoglobin of this amphibian has unusual properties which may help the animal to extract oxygen from a low pH environment. The molecular adaptation exhibited by *Amphiuma* haemoglobin is a pH dependence of oxygen binding that is reversed relative to that of most haemoglobins. Low pH brings about a large increase in the oxygen affinity of stripped *Amphiuma* haemoglobin. This negative Bohr effect indicates that protons are bound as oxygen is bound. This is in contrast to the release of protons that usually accompanies oxygenation. New Bohr groups must be present in this amphibian haemoglobin, or the normal Bohr groups must be drastically modified. From a physiological viewpoint it is of interest that the Bohr effect *in vitro* is subject to regulation by organic phosphate cofactors.

Haemolysates from the two *Amphiuma* studied gave single somewhat broadened bands on polyacrylamide disc gels, indicative of a single major haemoglobin. There were

no minor components, even on overloaded gels. Haemoglobin solutions were prepared by the ammonium sulphate procedure and stripped of organic and inorganic ions by treatment with a mixed-bed ion exchange resin¹. Oxygen equilibria were done spectrophotometrically using a tonometric procedure², and flash photolysis and rapid mixing experiments were done as described before³.

Figure 1 shows the large negative Bohr effect which characterises oxygen binding to stripped *Amphiuma* haemoglobin. Figure 1 also shows that 0.3 M NaCl lowers the oxygen affinity, decreases the pH dependence and increases cooperativity. Similar results are obtained with *Amphiuma* haemoglobin in 0.2 M inorganic phosphate. Organic phosphates cause an even greater decrease in oxygen affinity, although they do not appreciably alter the degree of cooperativity. Table 1 summarises these results and presents data on human haemoglobin A (HbA) for comparison. There are significant quantitative differences between HbA and *Amphiuma* haemoglobin

Table 1 Effects of anions and pH on the oxygen affinities of stripped preparations of *Amphiuma* haemoglobin at 20 °C in 0.05 M bis-Tris or Tris buffers (except for experiments in 0.2 M phosphate)

Haemoglobin	Cofactor	P_{50} (mmHg)		Bohr effect ($\Delta \log P_{50}$ (pH 7-8))
		pH 7	pH 8	
<i>Amphiuma</i>	none	4.0	8.3	-0.32
	0.3 M NaCl	5.9	10.2	-0.24
	0.2 M phosphate	5.1	8.9	-0.24
	2,3-diphosphoglycerate	6.7	—	—
	adenosine triphosphate	17.4	12.3	+0.15
	inositol hexaphosphate	20.0	13.5	+0.17
HbA	none	2.0	0.5	+0.56
	1.0 M NaCl	7.9	3.1	+0.41
	0.2 M phosphate	12.6	3.5	+0.55
	2,3-diphosphoglycerate	12.6	3.1	+0.60
	adenosine triphosphate	10.0	2.2	+0.66
	inositol hexaphosphate	38.9	11.5	+0.56

Data on human haemoglobin (HbA) are presented for comparison.

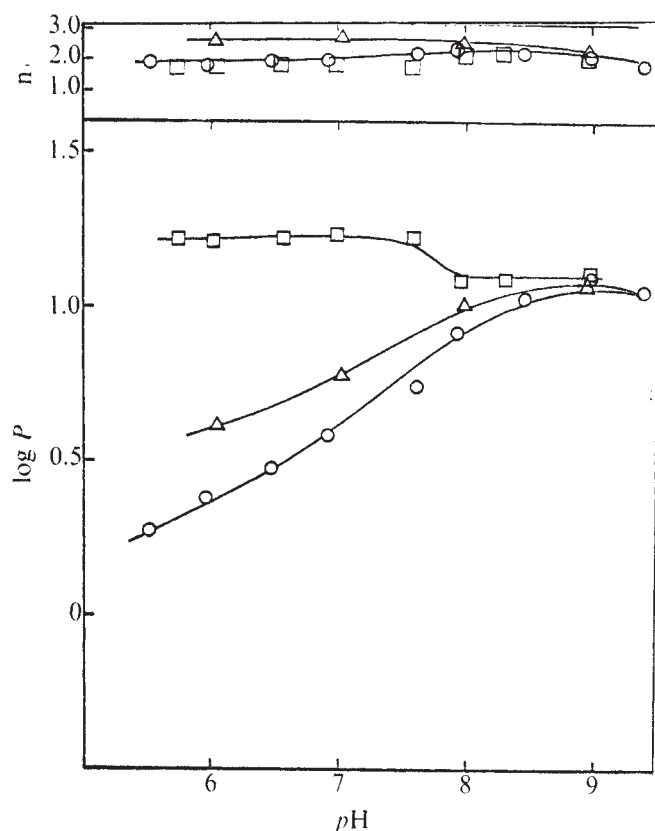


Fig. 1 Effect of pH and cofactors on the oxygen affinity and cooperativity of *Amphiuma* haemoglobin ($\sim 60 \mu\text{M}$ in haem) in 0.05 M bis-Tris or Tris buffers at 20 °C. Solutions of stripped haemoglobin were examined in the absence of cofactors ($\circ$), with 0.3 M NaCl (Δ), and with 100-fold excess of ATP:tetramer ($\square$). Oxygen pressures measured in mmHg.

in the relative effectiveness of various organic phosphates.

Enzymatically modified haemoglobin has been used to probe the mechanism of ligand binding. The properties of HbA after digestion with carboxypeptidase A and B, in particular, helped establish the important role played by its carboxyl-terminal residues⁴⁻⁶. Properties of carboxypeptidase A and B digests of *Amphiuma* haemoglobin, prepared as described elsewhere⁷, are shown in Fig. 2 and Table 2. The functional properties of the digestion products differ from those of carboxypeptidase digests of HbA. Interpretation of these differences is difficult because there are no structural data available on *Amphiuma* haemoglobin. We presume it to be an $\alpha_2\beta_2$ tetramer largely by analogy with other amphibian haemoglobins, and in part because of the identity of the amino acids released as the result of carboxypeptidase digestion^{8,9}. Carboxypeptidase B digestion of HbA removes arginine from its alpha chains. Similarly, arginine is the only residue released from a comparable digest of *Amphiuma* haemoglobin. Digestion with carboxypeptidase A causes $\beta 146$ histidine and $\beta 145$ tyrosine to be cleaved from the C-terminus of the beta chain of HbA. A comparable digest of *Amphiuma* haemoglobin releases only tyrosine.

The $\beta 146$ histidine of HbA is thought to be responsible for much of the positive Bohr effect of HbA^{4,5}. Consequently, absence of C-terminal histidine in *Amphiuma* haemoglobin may help explain its altered pH dependence. It is interesting that stripped tadpole haemoglobins show negative Bohr effects even though they possess a C-terminal histidine⁹. Amphibian haemoglobins typically have

low Bohr effects⁸, and absence of the C-terminal histidine in *Amphiuma* haemoglobin may therefore only accentuate a common divergence from mammalian haemoglobins.

The $\beta 145$ tyrosine of HbA is thought to play a major role in stabilisation of the low affinity (T) conformation¹. Removal of this residue is responsible for the high affinity and lack of cooperativity exhibited by HbA after digestion

a moderate increase in affinity and a decrease in cooperativity. In *Amphiuma* haemoglobin the C-terminal arginine is much more important, as demonstrated by the greatly increased oxygen affinity and loss of cooperativity which accompany digestion with carboxypeptidase B. It is remarkable that this non-cooperative digestion product has a strong negative Bohr effect. In the presence of inositol hexaphosphate, however, the carboxypeptidase B digest of *Amphiuma* haemoglobin shows a positive Bohr effect, comparable to that of HbA.

The kinetics of ligand binding by *Amphiuma* haemoglobin differ greatly from those of most other haemoglobins in that the typical pH sensitivity is reversed. For most haemoglobins the rate of oxygen dissociation is much faster at pH 7 than at pH 9, while the rate of ligand combination is slower at pH 7 than at pH 9. These kinetic factors work together in generating the positive Bohr effect of most haemoglobins. In contrast, Fig. 3 shows that low pH favours a slow rate of oxygen dissociation and a fast rate of CO combination for stripped *Amphiuma* haemoglobin. The pH dependence of these processes adequately accounts for the negative Bohr effect (Fig. 3). In accord with the equilibrium data, adenosine triphosphate (Fig. 3) alters these kinetic processes at neutral pH, with little effect at high pH.

At neutral pH the time course of CO binding to a concentrated solution of HbA shows an increasing rate as successive ligands are bound, indicative of cooperative subunit interactions¹. Similarly, the apparent rate constants increase 1.5–2-fold during the process of CO binding to *Amphiuma* haemoglobin. Flash photolysis of *Amphiuma* haemoglobin at low heme concentration ($\sim 0.5 \mu\text{M}$) gives no indication of quickly reacting material, and the autocatalytic character of the time course is maintained. This suggests that liganded *Amphiuma* haemoglobin does not readily dissociate into non-cooperative subunits. It will be of considerable interest to determine if pH or cofactors affect the state of aggregation of the protein. The time course of O_2 dissociation from stripped *Amphiuma* haemoglobin was also somewhat autocatalytic, although this was not as pronounced as in the kinetics of CO combination.

The foregoing serves to illustrate the unusual pH sensitivity of *Amphiuma* haemoglobin. Bohr effects that are low or slightly negative have been reported for haemoglobins from a number of other amphibians⁹, and we suspect that this characteristic may be a molecular adaptation to meet respiratory requirements during hibernation. None of the amphibian haemoglobins so far investigated has a negative Bohr effect as pronounced as that of *Amphiuma* haemoglobin, and no other cases of reversed pH sensitivity of both CO combination kinetics and O_2 dissociation kinetics have been reported.

The flexibility of haemoglobin function is brought into focus by this study by the fact that homotropic and heterotropic interactions can vary independently of one another. This is significant because homotropic and heterotropic interactions are tightly linked in normal human haemoglobin. Our functional studies with *Amphiuma* haemoglobin illustrate that the Bohr effect can be positive or negative without appreciable alteration of cooperative

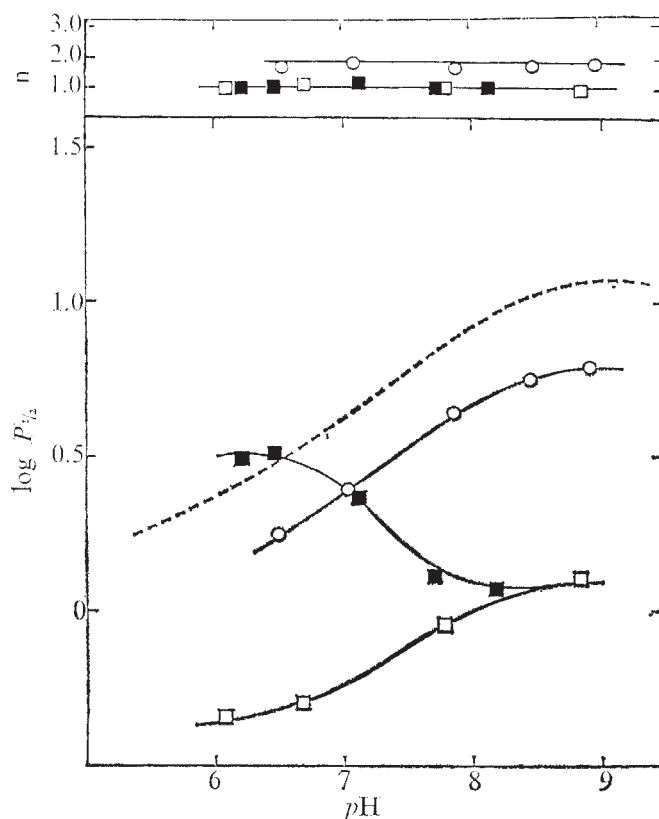


Fig. 2 Effect of pH on the oxygen affinities of carboxypeptidase A (○) and carboxypeptidase B (□) digests of *Amphiuma* haemoglobin. The effect of inositol hexaphosphate (■) on the CPB-digested material is also shown. Stripped *Amphiuma* haemoglobin ---. Other conditions as in Fig. 1.

with carboxypeptidase (refs 4–6). A Carboxypeptidase A digestion of *Amphiuma* haemoglobin does not eliminate cooperativity or greatly increase its oxygen affinity, which suggests that the C-terminal tyrosine of this haemoglobin is not as important in stabilisation of its low affinity state. Sensitivity to organic phosphates is maintained by the carboxypeptidase A digest and the Bohr effect is rendered positive by the addition of adenosine triphosphate or inositol hexaphosphate.

The $\beta 141$ arginine of HbA is thought to contribute to stabilisation of the low affinity (T) conformation by formation of ligand-linked salt bridges¹⁰. Loss of this residue after carboxypeptidase B digestion of HbA causes

Table 2 Effects of anions and pH on the oxygen affinities and cooperativity of stripped preparations of chemically modified forms of *Amphiuma* haemoglobin. Data collected at 20 °C in 0.05 M bis-Tris or Tris buffers

Modification	Cofactor	$P_{1/2}$ (mmHg)		$n_{1/2}$
		pH 7	pH 8	
Carboxypeptidase A digest (des Tyr)	None	2.5	4.42	1.7
	Adenosine triphosphate	7.4	—	1.5
Carboxypeptidase B digest (des Arg)	None	0.6	1.0	1.0
	Adenosine triphosphate	1.7	1.0	1.0
	Inositol hexaphosphate	2.6	1.2	1.0

interactions and that these changes in *pH* sensitivity can occur even in the absence of cooperative oxygen binding. In *Amphiuma* haemoglobin the separation of heterotropic and homotropic interactions is much more evident than in most other haemoglobins. Further study along these

Bonaventura as an Established Investigator of the American Heart Association.

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Errata

In 'Release of substance P from isolated nerve endings' by Christina Schenker, Edmund A. Mroz and Susan E. Leeman (*Nature*, **264**, 791, 1976), line 9 should read . . . not be released by high K^+ in the presence of Ca^{2+} . . .

In 'Significant journals of science' by E. Garfield (*Nature*, **264**, 609-15), on page 610, in Fig. 1a, the headings of the three columns to the right of the column of journal titles should read D, E and F. On page 612, in Fig. 2a, the column headings A, B and C should appear above the three columns on the left. On page 614, in the legend for Fig. 3, the definition of column D shown read "number of 1974 articles".

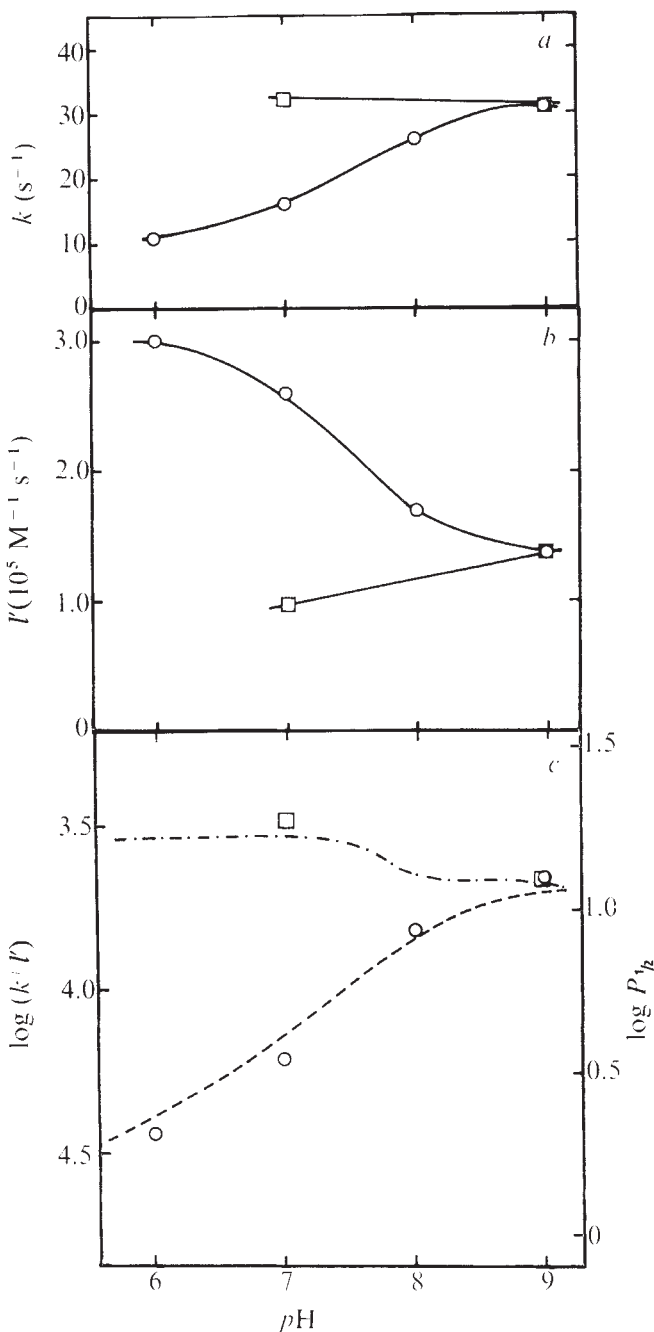


Fig. 3 Effect of *pH* on the apparent velocity constants at 20°C for oxygen dissociation (a) and CO combination (b) for stripped *Amphiuma* haemoglobin (○) and for the haemoglobin in the presence of 1 mM ATP (□). c, The log of the ratio of the kinetic constants (symbols) is compared to the equilibrium data of Fig. 1 (dashed lines).

lines should contribute to our understanding of the flexibility of haemoglobin function in response to environmental pressures.

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reviews

Hard reason and deep humanity

J. W. S. Pringle

Insects and the Life of Man: Collected Essays on Pure Science and Applied Biology. By Sir Vincent Wigglesworth. Pp. 217. (London: Chapman and Hall, 1976. Distributed in the USA by Halsted Press, a Division of Wiley: New York.) Paperback £3.25; hardback £6.

THIS book is a collection of essays or addresses published over a period of nearly forty years. Many of them are concerned with aspects of applied entomology and its relation to 'pure' science, but the life of man means more than just his material needs. So it is appropriate that the volume includes some of the contributions which Professor Wigglesworth has made to the history and philosophy of science and, in the widest sense, to its art.

The introductory chapter gives a guide to what follows. The earliest contribution is an article published in 1935 on malaria in Ceylon, which may well have stimulated the successful WHO campaign after the war. Wigglesworth did not foresee the problems that would arise from the resulting population explosion, but he did clearly forecast in 1945 the ecological effects that would result from widespread use of DDT. By 1951 he was acknowledging that "there can be no immediate prospect of abandoning the use of insecticides" but was pleading for more knowledge, with which "we should be able to devise methods of cultivation which would favour the crop and discourage the insect pest to the point where a satisfactory balance is established". This was well before the invention of "integrated control".

The essays are not primarily autobiographical, but chapter 12—an address to XIIth International Congress of Entomology in 1964—does start with a brief summary of Wigglesworth's career. Few people know that the *Rhodnius* cultures now so widespread in biological laboratories derive from a small stock imported from Venezuela about 1924 and laboratory-bred ever since. There follows a review of fifty years' progress in many fields of insect physiology since 1912—"impressive examples of the potential contribution of insect physiology to general biology". Yet, "I believe that the great era of insect physiology is yet to come": with the fertile observation that meta-

morphosis is a genetic switch, a step-wise activation of particular components of the genetic code, and that because in insects the switch is controlled by a single chemical, the juvenile hormone, "there is no more promising medium than the insect, in which this problem can hopefully be studied".

There is a stimulating essay (given to the British Association in 1948) on the history of entomological studies in Britain and the relationship between applied entomology and research. I particularly like the remarks about administration: "The keynote [of administration] should be the safeguarding and encouragement of research. Anyone who has tried both knows that administration is so immeasurably easier than research that it becomes the line of least resistance, and that is why research needs encouragement". Entirely historical is a sketch of the contributions of Sir John Lubbock (Lord Avebury) to insect physiology, "to put the record straight". "If Lubbock had held an academic appointment, he would have been accorded a high place among the biologists of the nineteenth century. But, like Darwin, he was an amateur; and unlike Darwin, he devoted only a minute fraction of his time to scientific pursuits".

Chapter 13 reprints a remarkable article on the epidermal cell, which is often overlooked since it was published in a *Festschrift* after delivery as a lecture to the Royal Society. The diversity of functions which these cells

can perform is one of the most remarkable phenomena in the whole of biology. There is material here for a generation of PhD theses.

The man behind the words comes through most clearly in chapter 16 (Wordsworth and Science) and in chapters 4 and 17 (Science, Pure and Applied; The Religion of Science). He admires Wordsworth "who, standing at the threshold of the Industrial Revolution . . . can compare with true scientific objectivity, though with all a poet's feeling, the evil of the factory child with the grinding penury of rural England" and who can, in 1810, find a solution and a hope in "a System of National Education established universally by Government". In chapters 4 and 17, Wigglesworth sets out clearly his faith, on which science is founded and by which it lives. "Without a deeply rooted instinctive belief in the existence of laws which reign throughout nature, the incredible labours of scientists would be without hope". Some rude things are said about philosophers, but the lecture was "anti-philosophical only to the extent that it takes the view that science and idealist philosophy have different ground rules and therefore cannot play together". The picture is of a man of hard reason, coupled with deep humanity.

I have only sampled some of the gems in this book. □

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Epidemics and pestilences

Insects and History. (The World Naturalist Series.) By J. L. Cloudsley-Thompson. Pp. x+242. (Weidenfeld and Nicolson: London, 1976.) £8.50.

THIS is a history book about pestilences, from the dawn of history to the present day. It is vividly written and well documented. Much of it makes horrifying reading. As the story progresses it seems to support the popular view that great epidemics are a thing of the past. But that past is not so very distant: the influenza pandemic of 1918 is thought to have resulted in 20 million

deaths—a larger number than any other pestilence in history. The chapter headings read like an account of medical entomology. But the value and interest of the book is enhanced by the large amount of space given to smallpox, scurvy, syphilis, famine and, of course, to the mixed infections and unidentified diseases which defy diagnosis at this distance in time.

The author is cautious in his attributions of historic change to specific insect-borne diseases. The well known idea of W. H. S. Jones that the loss of vigour among the Greeks when the power of Rome was increasing, was the

result of malaria. But the vigour of a people can be sapped from other causes; and when malaria does increase it is not always easy to distinguish cause and effect. Did the collapse of the Sinhalese civilisation in Ceylon result from the introduction of malaria? Or did malaria increase because the repeated Tamil invasions from the mainland disrupted the elaborate system of irrigated agriculture established by the Sinhalese. After all, it was probably improved and intensive agriculture which dispelled malaria from East Anglia in the last century. There seems little doubt that malaria in the Pontine marshes was a real factor among the epidemics which destroyed successive armies invading Rome. But in the inverse relationship pointed out by Celli between agricultural productivity in the Roman Campagna and the incidence of malaria, it may well have been intensive agriculture which was controlling malaria in ancient times as well as in the time of Mussolini.

It is a popular idea that malaria and swamps go hand in hand, and nothing is said in this book to dispel this belief. It happens to be true for much of Europe; but it is the reverse of the truth in most of the tropics, where the Anopheline mosquitoes which infest swamps and paddy fields are usually not carriers of malaria at all. The roadside puddle and the grassy streamlet are infinitely more dangerous. It came as a surprise to all sides in World War I to discover that in the Mediterranean area the mountain streams were often the major source of malaria.

This is a book about epidemics and pestilences, it is not about medical entomology. The insect carriage of disease is a subtle matter. Even the subtleties that are known scarcely appear in this book; and there is much that is not known. The author makes no pretence that the epidemiology of infectious diseases is a simple matter; he could hardly have covered the complexities of insect transmission as well as the rich history of epidemics that he provides. When the International Health Board of the Rockefeller Foundation was set up, it decided to make a big splash by tackling a project which it knew would be successful. General Gorgas had saved the labour force of the Panama Canal from yellow fever by first-class organisation of simple procedures. The International Health Board would banish yellow fever from the New World. That was in about 1912. In 1917 they claimed complete success, but a year or so later a small outbreak occurred and they had to do it again. And so it went on for years, until it was discovered by Soper in 1935 that the yellow fever virus was being maintained in the

Amazon jungle by forest mosquitoes feeding on monkeys in the tree-tops. When small clearings were made men were infected and returned to the towns, where they started epidemics carried by the urban mosquito *Aedes aegypti*. The Rockefeller Foundation had been unlucky; they had chosen a disease the eradication of which was impossible.

The author is an assiduous quoter of chapter and verse. There are times when the reader becomes tired of the repetitive statistics of horror. The effect, however, is cumulative, and the docketing of the fate of successive naval expeditions from the sixteenth century onwards, when faced with malaria, yellow fever, scurvy and the rest, bring home the facts in a way that no general account could do. The same applies to the figures for the incidence of typhus deaths in the wars of the eighteenth and nineteenth centuries. We all know that until recently the major cause of death in war was epidemic disease. But when the figures

are set out as here in detail, they are staggering.

In a concluding chapter the author deals with insects harmful to agriculture, concentrating his attention on the plague locusts which, as a major cause of famine, contribute greatly to the incidence of epidemic disease in man. He sketches also the former role of insects in commerce—as a source of dyes, waxes and lac, honey (for long the main source of sweetness) and silk, which played such an important part in the world trade of ancient China. All these products have receded in importance in modern times, but their impact on economic history as here recorded is fascinating.

Altogether this is a book that is full of intriguing information which cannot fail to interest both the general reader and the informed entomologist.

V. B. Wigglesworth

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Man's ectoparasites

Insects, Hygiene and History. By J. R. Busvine. Pp. 262. (Athlone: London, 1976. Distributed by Tiptree Book Services: Tiptree, Essex, and Humanities: New Jersey.) £6.95.

MOST of us who have lectured in medical entomology have tried to enliven our discussion of human ectoparasites by including a few anecdotes such as that demonstrating the holiness of St Thomas of Canterbury, whose garments, after his murder in the cathedral, "boiled over with lice like water simmering in a cauldron". This was, of course, in an age when cleanliness was considered to be the antithesis of godliness. We realised that there was a wealth of entertaining and instructive fact and fiction buried in the literature from the times of classical antiquity to the present day. A few writers—for instance, Hans Zinsser in his famous book *Rats, Lice and History*—have hinted at the richness of the information waiting to be revealed by some student of the byways of parasitology, but others, up till now, have not had the patience to follow his lead.

At last Professor J. R. Busvine has accepted this implicit challenge, and has written the first comprehensive account of the history, and the mythology, of man's ectoparasites. He has produced a most entertaining text, full of tales of observation and of misunderstanding, of ribald verse and donnish pornography. Future lecturers



Hair hygiene to eradicate lice

Horace Smith (1801)

will have no excuse if they make parasitology a dull topic.

This book has clearly been compiled during a lifetime study of insects and disease. James Busvine is not primarily a historian although he has done his historical research thoroughly and competently. He has systematically noted down each reference he has come across, sometimes by chance, often only after considerable searching, during the course of his major studies. But the great value of the book is that its author has himself contributed substantially to modern knowledge of its subject, so that he relates the historical observations, with all their curiosity, to the situation as understood by modern science.

This book is, however, more than an entertaining account of man's frailties. It shows how science progresses, and how this progress is often delayed. It is

often surprising to find that the same person could make accurate observations and draw the correct conclusions from them, and at the same time seem to be quite blind in another, though similar, field. Thus Aristotle was in some ways the father of scientific zoology, and many of his descriptions of animals and plants need little modification today. Yet, although he described the nit (the egg of the louse), he was convinced that it had no generative function, and that these insects arose only from the putrefaction of human flesh. This view was held for centuries, and to deny it could lead to a charge of heresy by the mediaeval church. Before the invention of the microscope naturalists could be excused for many misunderstandings, but it is difficult to comprehend why they were so badly mislead regarding this parasite, which can be so easily studied throughout its life history with the naked eye.

But the situation may not have changed as much as we may like to think. Scientists and clinicians still find

it difficult to accept new evidence and relinquish long-held beliefs. I well remember how incredulous some dermatologists were when, during the last war, we demonstrated that the itch mite, the cause of the disease of scabies, was usually transmitted by close personal contact and seldom by blankets and other inanimate objects. One leading physician stated publicly that these scientific experiments were all very well, but he *knew*, from a lifetime of clinical experience, that blankets and other fomites were of major importance. Fortunately he could not call on the inquisition to stifle our results, which were soon accepted by his colleagues and by the Ministry of Health, so that treatment was improved and the disease was, temporarily, nearly eradicated in Britain.

Kenneth Mellanby

Kenneth Mellanby was Reader in Medical Entomology in the University of London (1945-47) and Professor of Parasitology in the University of Ibadan (1948-53).

How behaviour works

The Hungry Fly: A Physiological Study of the Behaviour Associated with Feeding. By V. G. Dethier. Pp. 489. (Harvard University: Cambridge, Massachusetts, 1976.) £20.40.

THE appetites for sex and food are the two most important driving forces in animal behaviour, the one to maintain the species and the other to enable the performance of the first. Research on these appetites is therefore central to the study of behaviour. Most of our knowledge of the mechanisms involved in the appetite for food derives from work on the rat—an animal whose mentality is perhaps dangerously too near our own for complete objectivity. Some 30 years ago Dethier chose to work on the hunger of a simpler organism, the blowfly, with whom he and his students were less likely to identify. The result has been a volume of research scarcely less impressive than that on the rat, and in some respects much better in quality, notably on the electrophysiological correlates of taste and feeding.

The Hungry Fly is a kind of autobiographical account of these 30 years of research. It is difficult to categorise precisely, since the author seems not quite to have made up his mind whether to write a popular text or an advanced treatise. Thus, each chapter opens with a few rather elementary pages, some sprinkled with statements

that will seem either banal or erroneous to the professional biologist; but then plunges into the technicalities of chemosensory transduction or perseverating central excitatory states. The professional may be irritated by the introductions, the lay reader surely rapidly lost by the rest.

As one reads through the minutiae of experiments carried out in the 1950s that have since been superseded, one does begin to wish that the account was slightly less full. The book could surely have been slimmer, cheaper, and more assimilable if these false trails and some of the other fine detail had been omitted. Chapter 12, for example, describes at great length the neuro-

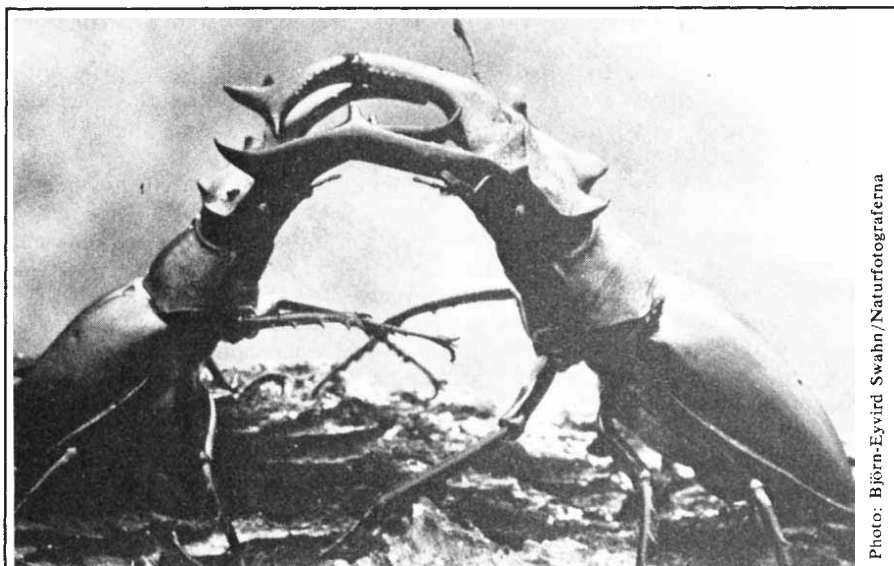
anatomy of the fly's brain with the aid of over 40 light micrograph serial sections (incidentally, without indicating scale, section position or thickness) and then ends frustratingly by saying that nothing whatever is known about the functional organisation of the dipterous brain.

Dethier uses the Lorenzian concepts of 'appetitive behaviour' and 'consummatory act' throughout his book, but the reader should not be thereby misled into thinking that he is still of that school of behaviourists who believe in these psychological notions of drive and motivation. His last chapter, perhaps the best in the book, spells out in brief, eloquent simplicity why there is no need to invoke these anthropomorphisms to explain the workings of behaviour, and why there is no need to consider the behaviour of the rat as qualitatively different from the simpler behaviour of the insect.

In effect, the book is a three-fold filling out and up-dating of Dethier's long 1969 review on the same subject. He says it is not about chemoreception and neurophysiology (though much of it is), but is a description of behaviour in terms of physiological mechanisms. In this respect it is a superb account, and will surely remain a classic on the shelves of behaviourist-physiologists for many years to come. It will probably also provide the more general biologist with insights into how behaviour works and what it is made of. Whether a wider public will be very much enlightened by it is less clear.

John Brady

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Fighting stag beetles. Taken from *The Secret Life of Insects* by P. Passarin d'Entrèves and M. Zunino. Pp. 378. (Orbis: London, November 1976.) £8.95.

Photo: Björn-Eyvind Swahn/Naturfotografarna

Economic geology and tectonics

Metallogeny and Plate Tectonics. Edited by D. F. Strong. Pp. vii+660. (Geological Association of Canada: Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, 1976.)

In some areas, the plate tectonic bandwagon must be approaching its elastic limit as more and more ideas and interpretations are heaped on it, yet surprisingly little attention had previously been paid to corollaries of this model for economic geology and Precambrian tectonics. Major progress in both aspects have now been made with the almost simultaneous publication of the proceedings of two separate NATO conferences—B. F. Windley's *The Early History of the Earth* (Wiley, 1976) and D. F. Strong's *Metallogeny and Plate Tectonics*. The latter comprises 26 papers and six abstracts concerned mostly with either general concepts or specific locations, with the main emphasis on attempts to interpret different metallogenies in terms of the current plate tectonic framework. The standard is inevitably variable, but generally very good. The papers are in English, with both English and French abstracts, and it is a credit to the editor that his work on the 'foreign' contributions is undetectable yet must be there for them to flow as well as they do. Similarly the editor must have worked hard to keep out too many pictures of subduction zone models which, although the dominating figure, do not obtrude.

The production is generally good, the only major flaw being that there is no index. This omission is particularly serious for such a heterogeneous collection of papers which, although grouped into sections, contain numerous cases of partial or even complete discussion of features which belong in other sections. This is further aggravated by the order in which the sections are presented: metal deposits in convergent plate margins, intraplate environments, orogenic belts and ores of Precambrian age. This order presumably reflects the fact that orogenic belts contain both subduction and intraplate ore bodies, and Precambrian deposits may contain all forms and also be characterised by modified plate tectonic models resulting from, for example, the much higher average geothermal gradients of Archaean times. Nevertheless, present day subduction zones, such as the Andes and Japan, which form the majority of the areas discussed under convergent zones, also contain pre-existing ore bodies in

the same way as the Canadian cordillera, which are discussed under orogenic belts. Such difficulties in grouping the papers would not have mattered so much if the whole volume was indexed, even in a coarse fashion. Under the circumstances, it would have been better to commence with accretion zone deposits, and then have orogenic belts following convergent zones.

Most of the early proponents of metallogeny and plate tectonics have contributed to this publication, but it is significant that the 'grey-beards', in this subject, such as R. H. Sillitoe, must be considered those who published on the subject in or before 1973, and sufficient credit is still not given to those geochemists who determined the zonations in island arc environments well before the discovery of plates. Those papers by E. Horikoshi (north-eastern Japan), A. Y. Glikson (Archaean to early Proterozoic Shield structures), T. Sato (green tuff province, Japan), F. J. Sawkins (massive sulphides), R. A. Stacey (Canadian cordillera) and H. S. Swinden and D. F. Strong (Appalachians, North American cordillera, south-eastern Australia) are particularly interesting. Others are disappointing rather than unimportant. For example, that by P. Laznicka (lead deposits on global plate model) could have been of major significance, but is marred by dogmatic classification of specific ore deposits—such as the Red Sea as intra-continental rather than an incipient ocean rise, the southern European Permo-Triassic deposits as a trailing

edge, and so forth. This may all be correct but is certainly arguable, yet the final conclusions are quoted to 0.01%; and one major environment, with 37.69% of world reserves, occurs in trailing margins—an environment which seems to be defined as one into which sedimentary ores are placed together with those which are not obviously in any other category. The conclusion should be that some one-third of the world's lead occurs in sedimentary forms of uncertain origin.

The general lack of consideration of sedimentary ores is understandable, although disappointing. There is evidence that most of these have been strongly influenced by palaeoclimatic conditions and hence palaeolatitudes. As this is determined by the motions of the plates, these ores are as related to plate tectonic motions as subduction zone metals. Other gaps, however, include kimberlites, which are not merely important economically but have major implications for upper mantle evolution. Nevertheless this publication is obviously essential to even the smallest geological library. The hope is now that some future NATO conference will turn to consider energy resources in the same context. The seafloor conveyor still has some way to go before all of the ideas have been sweated out of its subducting melange! **D. H. Tarling**

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Opisthobranch molluscs

British Opisthobranch Molluscs. (Synopsis of the British Fauna, No. 8.) By T. E. Thompson and G. H. Brown. Pp. 203. (Academic: London, New York and San Francisco, 1976.) £3.50.

THIS is a very welcome addition to the recent series of Synopses of the British Fauna. The book is well designed and attractive to look at. There is less restriction on size than in previous numbers and this has enabled the authors to have, for most species, a large drawing occupying one page and on the opposite page a brief but adequate description of external features necessary for identification, notes on biology and worldwide distribution. Technical terms have been kept to a minimum and the introductory pages and descriptions are designed to cater for the non-professional zoologist.

The complementary talents of both authors are well exhibited. Gregory H. Brown's drawings set forth the

salient features of each species and the life-like posture. T. E. Thompson, a well known specialist on opisthobranchs and an aqualung enthusiast, is familiar with the habitats of many species and has examined nearly all the 135 which are described. When opisthobranchs have to be identified it is now no longer necessary to search the classical works and paraphrase the language of nearly 150 years ago. In this synopsis concise keys lead to family and species. Ideally such attractive molluscs would be depicted in colour, but the cost is prohibitive. The single plate that is provided, although valuable in displaying their beauty of form, whets the appetite for more.

In such a detailed compilation it is difficult to prevent a few misconceptions: the eyes of *Stiliger bellulus* are lacking in the description and the figure; the dorsal surface of *Limapontia capitata* is figured with a pale cross, but this is of rare occurrence.

V. Fretter

V. Fretter is a former Reader in the Department of Zoology at the University of Reading, UK.

announcements

Appointments

Dr R. W. J. Keay has been appointed Executive Secretary of the Royal Society. A service of thanksgiving for Sir David Martin, the late Executive Secretary, will be held at St Columba's Church of Scotland, Pont Street, London SW1 on February 9, 1977 at 12.00 noon.

Professor J. H. H. Merriman has been appointed as Chairman of the National Computing Centre, Manchester.

Dr John A. Gray has been appointed as a member of the Advisory Council on Research and Development for fuel and power (ACORD).

Professor F. G. T. Holliday has been appointed as Chairman of the Nature Conservancy Council.

Mr Bryan Davies, MP, has been appointed as a member of the Medical Research Council.

Awards

Peter Dennis Mitchell is the winner of Brandeis University's sixth annual Rosenstrel Award. Dr Mitchell was awarded the prize of \$5,000 for pioneering work in cellular physiology.

Dr D. C. Gajdusek is the winner of the triennial prize of the Fondation de Physiopathologie Professeur Lucien Dautrebande. The award of B.Fr 700.000 is for work with therapeutic implications on human or animal physiotherapy. For further information contact Office de la Fondation, 35 Chaussée de Liège, 5200 Huy, Belgium.

Meetings

February 16, 2.00 pm, Fortress House, Savile Row, London W1. Council of Science and Technology Institutes Interdisciplinary Degrees and Institute Qualifications.

February 17, **Financial Control of Laboratory Animal Facilities**, London (Mr M. Robinson, Medical Research Council Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF. Tel: 01-643 8000).

March 23-24, **Fibre Reinforced Materials**, London (Institute of Civil Engineers, Great George Street, London SW1).

March 24-25, **EM Negative Staining: Viruses, Macromolecules and Membranes**, Hornchurch, Essex (J. R. Harrison, Biochemistry, North East London Polytechnic, London).

Person to Person

Inventory of unpublished documents in twentieth century physics. The Office for History of Science and Technology at the University of California, Berkeley, is undertaking a world-wide survey of archival holdings related to physics between 1900 and 1950. Of particular interest is documentation of contact between physicists and others outside the domain of academic physics. Readers with knowledge of (a) unpublished correspondence with physicists, particularly items in private hands or in archival collections associated primarily with non-physicists; (b) letters to or from a physicist published in journals or books not likely to be well known to historians of science; (c) archival holdings of the papers of little-known physicists, are asked to contact The Survey of Archives, c/o Office for History of Science and Technology, 470 Stephens Hall, University of California, Berkeley, California 94720, U.S.A.

Family of five want to exchange with another family a large London house (possibly and car) for Winnebago or equivalent (preferably and house) in California. For about 8 weeks from second week in July 1977. P. Newmark, *Nature*, 4 Little Essex Street, London, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

March 29, **Crop Protection**, Newport, Salop (E. Holden, Institution of Agricultural Engineers, West End Road, Silsoe, Bedford).

April 4-7, **Rock Engineering**, New-castle-upon-Tyne (Institution of Civil Engineers, Great George Street, London SW1).

April 11-13, **Colloid stability**, Lunteren, Netherlands (G. D. Parfitt, Tioxide International Ltd, Billingham, Cleveland, England).

April 14-15, **35th Annual Congress and Scientific Exhibition**, London (British Institute of Radiology, 32 Welbeck Street, London).

April 20-22, **3rd International Congress on Static Electricity**, Grenoble (Société de Chimie Industrielle, 28 rue Saint-Dominique 75007 Paris).

May 4-5, **International Symposium on Epidermological Evaluation of Drugs**, Milan (Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea, 62, 20157, Milano, Italy).

May 9-12, **Biological Solar Energy Conversion Systems**, Grenoble-Autrans (P. M. Vignais, DRF/Biochimie, C.E.N.-G., 85X, 38041 Grenoble-Cedex, France).

May 13-27, **Genetics and Molecular Biology of Tetrahymena**, Copenhagen, Embo course (F. Zeuthen, Biological Institute of the Carlsberg Foundation, 16 Tagensvej, DK 2200-Copenhagen N, Denmark).

May 17-20, **Brookhaven Symposium 29: Genetic Interaction and Gene Transfer**, New York (C. W. Anderson, Biology, Brookhaven National Laboratory, Upton, New York 11973).

July 5-7, **International Dynamic Mass Spectroscopy Symposium**, Salford (D. Price, Chemistry and Applied Chemistry, University of Salford, UK).

July 8-9, **Genito-urinary Trichomoniasis**, Paris (A. Fari, S.I.T.G.U. 5 Bld de Strasbourg, 75010 Paris, France).

July 18-22, **International Nutrition: Problems, Policies and Strategies**, Cambridge, Ma (Summer Session Director, Room E19-356, MIT, Cambridge, Ma 02139).

August 14-18, **4th International Workshop on Human Gene Mapping**, Winnipeg (J. L. Hamerton, 685 Bannatyne Avenue, Winnipeg, Manitoba, Canada).

September 13-15, **Ion-Ion and Ion-Solvent Interactions**, Oxford (Y. A. Fish, Chemical Society, Burlington House, London, W1).

September 19-21, **The 50th Anniversary of the Discovery of Electron Diffraction**, London (P. J. Dobson, Physics, Imperial College, London, SW7).

September 20-22, **Non-Linear Problems in Stress Analysis**, Durham (A. S. Kenkare (for Institute of Physics), Hatfield Polytechnic, Hatfield, Herts.).

October 10-14, **Soaps and Detergents**, Montreux (Karl Zilch, Emery Industries, Cincinnati, Ohio).

November 21-25, **Food Preservation by Irradiation**, Wageningen, Netherlands (IAEA, Kartner Ring 11, A1011 Vienna, Austria).

December 13-14, **Electrocrystallisation Nucleation and Phase Formation**, Southampton (Y. A. Fish, Chemical Society, Burlington House, London, W1).

Reports and Publications

UK & Ireland—December

- Medical Research Council: Laboratory Animals Centre. Manual Series No. 4: Laboratory Animal Houses—a Guide to the Design and Planning of Animal Facilities. By G. Clough and M. R. Gamble. Pp. iv + 44. (Carshalton, Surrey: MRC, Laboratory Animals Centre, 1976. Grats.) [212]
- The Hopeful Captive Customer. By Sir Arthur Hawkins. Pp. 8. London: Central Electricity Generating Board, 15 Newgate Street, EC1, 1976.) [212]
- Chemical Industries Association, Ltd. Activities Report 1975/1976. Pp. 16. (London: Chemical Industries Association, Ltd., 93 Albert Embankment, SE1, 1976.) [212]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 276, No. 943: A Discussion on the Assembly of Regular Viruses. Organized by A. Klug, FRS and P. J. G. Butler. Pp. 1–204. UK £17.05; Overseas £17.55. Vol. 276, No. 944: Organization of Motoneurons in the Prothoracic Ganglion of the Cockroach *Periplaneta Americana* (L.). By J. F. Iles. Pp. 205–219 plates 1–3. UK £14.5; Overseas £15.0. (London: The Royal Society, 1976.) [312]
- Office of Population Censuses and Surveys. Local Authority Vital Statistics: Vital Statistics for Administrative and Health Areas of England and Wales, 1974. (Series VS, No. 1.) Pp. 14. (London: HMSO, 1976.) 65p net. [312]
- The Declining Otter: a Guide to Its Conservation. By Angela King, John Ottaway and Angela Potter. Pp. 63. (Chaffcombe, Nr. Chard, Somerset: Friends of the Earth Otter Campaign, Yew Tree Cottage, 1976.) 65p + 10p p&p. [612]
- Agricultural Research Council Annual Report of the Institute for Research on Animal Diseases for 1976. Pp. 86. (Compton, Near Newbury: ARC Institute for Research on Animal Diseases, 1976.) [612]
- The Amateur Entomologists' Society. Leaflet No. 22: Collecting Lacewings. By Lt.-Col. F. C. Fraser. Revised second edition. Pp. 9. (Hanworth, Middx: Amateur Entomologists' Society, 355 Hounslow Road, 1976.) 60p. [712]
- Cholera in Africa: Diffusion of the Disease 1970–1975 with particular emphasis on West Africa. By Robert F. Stock. (African Environment Special Report 3.) Pp. 127. (London: International African Institute, in association with the Environment Training Programme, 1976.) \$4.50. [712]
- Ministry of Agriculture, Fisheries and Food. Agricultural Development Advisory Service. Science Service Annual Report 1975. Pp. xxi + 308. (London: HMSO, 1976.) £9 net. [812]
- Ardnamurchan: a Guide to Geological Excursions. By C. D. Gribble, E. M. Durrance and J. N. Walsh. Pp. ix + 122. (Edinburgh: Edinburgh Geological Society, c/o Grant Institute of Geology, West Mains Road, 1976.) £2. [812]
- Voice of the People: V 2 P—a Plain Man's Guide to Democracy. By Parnell Bradbury, based on an idea by Farel Bradbury. Pp. 12. (Ross-on-Wye: Hytadum, 1976.) 85p. [812]
- The British Library—Research and Development Reports. Report No. 5299 HC: Trends in Scholarly Publishing. Pp. iv + 87. (London: The British Library, Research and Development Department, Sheraton House, Great Chapel Street, W1, 1976.) £3.50. [812]
- Report on Fodder Maize in Ireland 1971–1975. Compiled and edited by M. Neenan. Pp. 41. (Dublin: Foras Talintais, 19 Sandymount Avenue, 1976.) £1.75. [812]
- The Royal Society. Scientific Research in Schools Committee: Report to Council 1976. Pp. 12. (London: The Royal Society, 1976.) [912]
- Office of Population Censuses and Surveys. Population Trends 6, Winter 1976. Pp. 56. (London: HMSO, 1976.) £2 net. [912]
- A Review of the Bony-Toothed Birds (Odontopterygiformes): With Descriptions of Some New Species. By C. J. O. Harrison and C. A. Walker. Pp. 62 + 10 plates. (Tertiary Research Special Paper No. 2.) (London: The Tertiary Research Group, 1976.) £3. [912]
- British Nuclear Forum. UK Nuclear Power Industry. Pp. 51. (London: British Nuclear Forum, 1 St. Alban's Street, SW1, 1976.) [912]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 276, No. 945: The History of the Mammal Fauna During the Ipswichian/Last Interglacial in England. By A. J. Stuart. Pp. 221–250. (London: The Royal Society, 1976.) UK £1.80; Overseas £1.85. [912]
- University of Nottingham. School of Agriculture Report 1975–1976. Pp. 171. (Sutton Bonington, Loughborough: University of Nottingham, School of Agriculture, 1976.) £1.70. [1312]
- Department of the Environment. Research Report 17: The Environmental Problems of Tailings Disposal at Metal Mines. Pp. iv + 33. (London: Department of the Environment, 2 Marsham Street, SW1, 1976.) £1.61 (postage paid). [1312]
- Tetrahedron Reports. No. 16: Ynamine: a Versatile Tool in Organic Synthesis. By J. Ficina. Pp. 38. £4.95; \$10. No. 17: The Organic Photochemistry of Benzene—I. By D. Bryce-Smith and A. Gilbert. Pp. 18. £4.95; \$10. No. 18: Studies on the Synthesis of Corrin and Related Ligands. By Robert V. Stevens. Pp. 14. £4.95; \$10. No. 19: New Horizons in Carbonyl Chemistry: Reagents for Nucleophilic Acylation. By O. William Lever, Jr. Pp. 29. £4.95; \$10. (Oxford and New York: Pergamon Press, 1976.) [1312]
- University of Cambridge. Annual Report of the Institute of Astronomy, 1975/1976. Pp. 20. (Cambridge: The University, 1976.) [1412]

- Nature Conservancy Council: Second Report Covering the Period 1 April 1975–31 March 1976. Pp. vi + 126 + 29 plates. (London: HMSO, 1976.) £1.75 net. [1412]
- The University of Hull. Annual Report of the Council and the Senate to the University Court, 1975/1976. Pp. v + 95. (Hull: The University, 1976.) [1512]
- University of Cambridge—Local Examinations Syndicate. School Examinations and Their Function. Pp. 16. (Cambridge: The University, 1976.) [1512]
- The British Library. Third Annual Report, 1975/1976. Pp. 48. (London: The British Library, 1976.) [1512]
- Department of the Environment: Central Unit on Environmental Pollution. Pollution Paper No. 9: Pollution Control in Great Britain: How It Works. (A Review of Legislative and Administrative Procedures.) Pp. 104. (London: HMSO, 1976.) £1.40 net. [1612]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 283, No. 1315: A Discussion on Valley Slopes and Cliffs in Southern England—Morphology, Mechanics and Quaternary History. Organized by A. W. Skempton, FRS and J. N. Hutchinson. Pp. 421–635 + plates 1–13. (London: The Royal Society, 1976.) UK £13.95; Overseas £14.45. [1612]
- Waste Management Advisory Council. Paper No. 3: An Economic Case Study of Waste Oil and Its Wider Significance. Pp. iii + 28. (London: HMSO, 1976.) 60p net. [1712]
- University of Nottingham. School of Agriculture Report 1975/1976. Pp. 171. (Sutton Bonington, Loughborough, Leicestershire: University of Nottingham School of Agriculture, 1976.) £1.70. [1712]
- The CEGB's Achievements and Prospects. By Sir Arthur Hawkins. Pp. 9. (London: Central Electricity Generating Board, 15 Newgate Street, EC1, 1976.) [1712]
- Geological Survey of Ireland. Guide Series No. 1: Geological Guide to the Dingle Peninsula. By Ralph R. Horne. Pp. 53. (Dublin: Ministry of Industry and Commerce, The Geological Survey of Ireland, 1976.) [1712]
- A Short Note on Elementary Particles. By John M. S. Speirs. Pp. 32. (Fleet, Hants: J. M. S. Speirs, 9, Peatmoor Close, 1976.) [1712]
- The Future of the United Kingdom Power Plant Manufacturing Industry—Report by the Central Policy Review Staff. Pp. xviii + 102. (London: HMSO, 1976.) £2.50 net. [1712]
- Report of the Centre for Overseas Pest Research, January–December 1975. Pp. vi + 142. (London: Centre for Overseas Pest Research, College House, Wrights Lane, 1976.) £2.75. [2012]
- University of Oxford. Report of the Committee to Review the Future of the Ruskin School of Drawing and Fine Art. Pp. 18. (Oxford: The University, 1976.) £1. [2012]
- Telecommunications Policy, Vol. 1, No. 1, December 1976. General Editor: Lawrence H. Day. Pp. 1–96. Annual subscription (4 issues) £30; \$78. (Haywards Heath, Sussex: IPC Business Press, Oakfield House, Perryman Road, 1976.) [2012]
- Proceedings of the Royal Irish Academy. Vol. 76, Section B, No. 26: The Stratigraphy of the Area Around Clonakilty Bay, South County Cork. By J. R. Graham and T. A. Reilly. Pp. 379–391 + plates 13 and 14. (Dublin: Royal Irish Academy, 1976.) 84p. [2112]
- Office of Population Censuses and Surveys. Statistics of Infectious Diseases: Notifications of Infectious Diseases in England and Wales, 1975. (Series MB2 No. 2) Pp. iv + 37. (London: HMSO, 1976.) 95p net. [2112]
- University of Oxford. Annual Reports, 1975/76. Pp. 21. (Oxford: The University, 1976.) £1.25. [2212]
- Chemicals 77: a Directory of Chemicals on the UK Market. Pp. 211. (London: Chemical Industries Association, Ltd., 93 Albert Embankment, 1976.) UK £8; Overseas £10. [2212]
- Computer Board for Universities and Research Councils: Report of the Computer Board for the period 1 April, 1975–31st March 1976. (Cmd. 6696.) Pp. ii + 19. (London: HMSO, 1976.) 45p net. [2912]
- International Union of Pure and Applied Chemistry. Inorganic Chemistry Division—Commission on Atomic Weights. Atomic Weights of the Elements 1975. Pp. 77–95. (Oxford and New York: Pergamon Press, 1976.) \$6. [2912]
- Tetrahedron Report No. 15: Catecholborane—a New Hydroboration Reagent. By Clinton F. Lane and George W. Kabalka. Pp. 10. (Oxford and New York: Pergamon Press, 1976.) £4.95; \$10. [3112]

Other countries—December

- United States Department of the Interior: Geological Survey. Professional Paper 915: Plutonic Rocks of the Santa Rita Mountains, Southeast of Tucson, Arizona. By Harald Drewes. Pp. v + 75. Professional Paper 680-F: Late Cenozoic Ostracoda from Midway Island Drill Holes. By John C. Holden. Pp. iv + 43 + 17 plates. (Washington, DC: US Government Printing Office, 1976.) [1112]
- National Museum of New Zealand. Records. Vol. 1, No. 8: Some Early Maori Wood Carving from Ruatuhuna, Urewera District, New Zealand. By Roger Neich. Pp. 127–142. Miscellaneous Series, No. 1: Marine Algae of the Kaikoura Coast. By G. Robin South and Nancy M. Adams. Pp. 67. Report of the Board of Trustees of the National Art Gallery, the National Museum, and National War Memorial for the year ended 31 March 1976. Pp. 32. (Wellington: National Museum of New Zealand, 1976.) [1112]
- Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 76–1C: Report of Activities, Part C. Pp. vi + 334. (Ottawa: Printing and Publishing, Supply and Services Canada, 1976.) \$6. [1112]

- World Health Organization. Technical Report Series. No. 592: Pesticide Residues in Food—Report of the 1975 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. Pp. 45. Sw.fr.6; \$2.40. No. 597: Epidemiology of Onchocerciasis—Report of a WHO Expert Committee. Pp. 94. Sw.fr.7; \$2.80. (Geneva: WHO; London: HMSO, 1976.) [212]
- Surveillance of Drinking-Water Quality. (Monograph Series, No. 63.) Pp. 135. (Geneva: WHO; London: HMSO, 1976.) Sw.fr.29; \$11.60. [312]
- Seminar on Science Publishing in India—Problems and Prospects. New Delhi, 18–19 November 1976: Abstracts of Papers. Pp. 62. Science Publishing—a Bibliography for Technical Editors. By S. Arunachalam, D. C. Goswami and P. S. Shankar. Pp. 21. (New Delhi: Publications and Information Directorate, Hillside Road, 1976.) [312]
- World Health Organization. Technical Report Series. No. 596: Application of Systems Analysis to Health Management—Report of a WHO Expert Committee. Pp. 69. (Geneva: WHO; London: HMSO, 1976.) Sw.fr.7; \$2.80. [612]
- Sick Fund of the General Federation of Labour in Israel. Kupat-Holim Year Book. Vol. 5. Pp. 205. (Tel-Aviv: The Family Physician, 1976.) [612]
- Annual Report of the Mauritius Sugar Industry Research Institute for 1975. Pp. 68. 15 statistical tables. (Rédut, Mauritius: Mauritius Sugar Industry Research Institute, 1976.) [612]
- Prime Ministers Office, Jerusalem. National Council for Research and Development. Seawater Desalination. (Proceedings of the Eleventh National Symposium on Desalination, Oholo, March 19–20, 1975.) Edited by David Hasson and Amiel Dickmann. Pp. 297. (Jerusalem: National Council for Research and Development, 1976.) [612]
- The Two Faces of Malnutrition. By Erik Eckholm and Frank Record. (Worldwatch Paper 9.) Pp. 63. (Washington, DC: Worldwatch Institute, 1976) Massachusetts Avenue, N.W., 1976.) \$2. [712]
- Skogshogskolan/Royal College of Forestry, Stockholm. Studia Forestalia Suecica, Nr. 134: Virkesdrivning inom Kramfors-delen av SCA 1933–1965/Logging and Transport in the Kramfors Forests of SCA 1911–1965. Av. Sven Embersten. Pp. 134. (Stockholm: Skogshogskolan/Royal College of Forestry, 1976.) [712]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 242: Mesozoic and Tertiary Rocks of Quatsino Sound, Vancouver Island, British Columbia. By J. A. Jezletzky. Pp. 243. (Ottawa: Information Canada, 1976.) \$7. [712]
- Food and Nutrition Strategies in National Development: Ninth Report of the Joint FAO/WHO Expert Committee on Nutrition, Rome, 11–20 December 1974. Pp. 64. £1.20. Pesticide Residues in Food: Report of the 1975 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. Geneva, 24 November–3 December, 1975. Pp. 45. £1. (Rome: FAO; London: HMSO, 1976.) [812]
- Environment Canada. Fisheries and Marine Service. Technical Report No. 635: Summer Distribution of Groundfish on the Scotian Shelf 1970–1974. By J. S. Scott. Pp. iv + 16. (St. Andrews, NB: Research and Develop. Directorate, Biological Station, 1976.) [812]
- A Selected and Partially Annotated Bibliography of Society, Ethics and the Life Sciences, 1976–77. Compiled by Sharon Solitto and Robert M. Veatch. Revised by Nancy K. Taylor. Pp. 82. (Hastings-on-Hudson: Institute of Society, Ethics and the Life Sciences, 1976.) [912]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Chemical Physics, 1975/76. Pp. 79. (East Melbourne: CSIRO, 1976.) [1012]
- Southern California Coastal Water Research Project. Annual Report for the Year Ended 30th June 1976. Pp. v + 263. (El Segundo, California: Southern California Coastal Water Research Project, 1500 East Imperial Highway, 1976.) [1012]
- United States Department of the Interior: Geological Survey. Professional Paper 576-D: Sedimentary Rock Alteration in the Slick Rock District, San Miguel and Dolores Counties, Colorado. By Daniel R. Shawe. Pp. v + 51 + 2 plates. Professional Paper 899: The Karst Landforms of Puerto Rico. By Watson H. Monroe. Pp. iv + 69. (Washington, DC: US Government Printing Office, 1976.) [1012]
- Australia: CSIRO. Central Information, Library and Editorial Section. Report 1973/1975. Pp. 76. (East Melbourne: CSIRO, 1976.) [1012]
- CERN: European Organization for Nuclear Research. CERN 76-02: Publications Covering Bubble Chamber Experiments Carried Out at CERN during the year 1975. Addendum to List of Publications CERN 76-02. Pp. v + 66. (Geneva: CERN, 1976.) [1312]
- The Walter and Eliza Hall Institute of Medical Research. Annual Review 1975/1976. Pp. 128. (Melbourne: The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, 1976.) [1412]
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